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TRANSACTIONS

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THE LIFE HISTORY AND TAXONOMIC POSITION OF *VENTURIA RUMICIS* (DESM.) WINT.

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(With 5 Text-figures)

The growth, development and life history of *Venturia rumicis* on its *Rumex* host and in culture are described. The fungus is homothallic, producing its perfect state on the living host and in culture; no asexual reproductive state has been found.

Centrum development in *Pleospora herbarum* (Pers.) Rabenh., *V. inaequalis* (Cke) Wint., *V. pirina* Aderh. and *Mycosphaerella maculiformis* (Pers.) Rabenh. is described and compared with that in *V. rumicis*. It is of the *Dothidea*-type in *M. maculiformis*, but of a *Pleospora*-type in the other four.

The classification of the ascostromatic Ascomycetes is considered, and in the light of this the taxonomic position of *V. rumicis* is discussed, with the conclusion that the correct name is *V. rumicis* (Desm.) Wint.

This work was undertaken in an attempt to clear up the confusion in the nomenclature of the fungus which has been known under two generally accepted names: *Mycosphaerella rumicis* (Desm.) Grove (1933) and *Venturia rumicis* (Desm.) Winter (1887). It was first recorded and described by Desmazières (1843) as *Sphaeria rumicis* and then by Cooke (1866) as *Sphaerella rumicis*. The two descriptions are almost identical; neither includes mention of spines or appendages on the fruit body nor of paraphyses in the fruit body cavity. The only difference is that Desmazières said the ascus wall was two-layered. Winter recorded spines on the fruit body and also internal paraphyses, and for these reasons placed the fungus in *Venturia*.

The first legitimate use of the combination *M. rumicis* was by Grove (1933); Lindau (1900) used this combination, without citing authorities, in discussing the relationship of the fungus to *Ovularia obliqua* (Cooke) Oud., and Oudemans (1905) referred to a combination *M. rumicis*, used by Johanson and Magnus in *Öst. bot. Z.* **6**, 1904, but in this journal no reference to the fungus was found (Wakefield, 1939).

Laibach (1921) recorded a further complication in the nomenclature of *V. rumicis*, in that Schröter (1897) and Migula (1912) named the fungus *Stigmatea rumicis*, a name which he favoured. Their reasons for adopting it are based on habitat and absence of spines from the fruit body. However, *S. rumicis* has never been adopted. The dimidiate fruit bodies of *Stigmatea* are so characteristic that, even in the more recent schemes of classification (Luttrell, 1955), this character separates *Stigmatea* from genera with a spherical fruit body.

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The combination *Spilosticta rumicis* was used by Sydow (1923), but his erection of the genus *Spilosticta* has not been generally accepted and *Spilosticta* is considered a synonym of *Venturia* (Korf, 1956).

On the continent *V. rumicis* has generally been adopted (Munk, 1957; Müller & Arx, 1950), while in British mycological literature *M. rumicis* has tended to be used (Grove, 1933; Bisby & Mason, 1940), and the name is still used in the official foray lists (*Trans. Brit. mycol. Soc.* **39**, 1956) which usually follow the check lists. However, Dennis (1960) lists the fungus as *V. rumicis*.

Another controversy has arisen over the association of *V. rumicis* with *O. obliqua*; the first report of it being the conidial state of *V. rumicis* was by Fückel (1869); Lindau (1900) and Langeron (1945) repeated this assertion. However, Laibach reported the perfect state of *O. obliqua*, found on the undersurface of *O. obliqua* lesions on overwintered leaves, to be a *Mycosphaerella*-type, quite unlike *V. rumicis*; he called it *Ovosphaerella lapathi*. He confirmed that it was the perfect state by obtaining a culture of *Ovularia obliqua* from a single ascospore. He also inoculated leaves with a suspension of asci and ascospores and obtained *O. obliqua* lesions within 12 days. The method used to obtain the suspension would seem to make it very difficult to ensure that no conidia of *O. obliqua* were present.

However, though the work of Laibach was not repeated, it was shown later that similarity of host is almost certainly the only connexion between *O. obliqua* and *V. rumicis*.

MATERIALS AND METHODS

All the fungi studied in this work were obtained in the grounds of The Royal Holloway College, Surrey: *V. rumicis* and *O. obliqua* from the living host, *V. inaequalis*, *V. pirina* and *M. maculiformis* from dead host material, and *P. herbarum* from a dead *Rumex* leaf. Centrum development was investigated by sectioning fruit bodies, both fresh and fixed, by hand or with a freezing microtome. Microtome sections were also made of wax-embedded material. Cotton blue in lactophenol proved the most satisfactory stain both for the study of host–parasite relationships and for investigations of development.

GROWTH, DEVELOPMENT AND LIFE HISTORY

V. rumicis causes a leaf-spotting disease of *Rumex* spp. and may also form elongated lesions on the stems and petioles. Very frequently the spots have a red border, which makes them distinctive and easily noticed. Red-bordered spots are not unique to *V. rumicis*; they are often formed by other *Rumex* leaf pathogens, and can sometimes be induced by wounding. In these red areas the sap of the cells is red. In the centre of a spot a brown area of dead leaf tissue contains the black fruit bodies of the fungus. The oldest and largest of these are in the centre of the spot, the others being arranged more or less concentrically. At the edge of a spot young fruit-bodies develop in leaf tissue which is still green; in fact, when the diseased leaf is beginning to turn yellow the area at the edge of infected spots often remains much greener than the rest of the leaf. Sometimes there are no fruit bodies at the centre and then the fruit bodies are arranged round a central area of dead tissue; a 'shot hole' type of spot is sometimes found when this has disintegrated.

Diseased leaves are found all the year round, the fungus seems to thrive especially in cool wet conditions, and the disease is least prevalent in hot dry weather.

The fungus has been recorded on most species of *Rumex*, but not on *R. acetosa* or *R. acetosella.* It is reported to be widespread in Europe (Lindau, 1897) and has been widely recorded in Great Britain.

V. rumicis is readily cultured and has continued to produce a perfect state through continued subculturing. Despite all attempts to induce asexual reproduction, no conidial state has ever been found.

Ovularia obliqua *and* V. rumicis

O. obliqua also causes a leaf-spotting disease of *Rumex* spp. and, like *V. rumicis*, has not been found on *R. acetosa* or *R. acetosella*. The spots, which may or may not be red bordered, are at first like those of *V. rumicis*, but gradually the diseased area widens and often, especially in moist conditions, may cover a large area of leaf. The dead leaf tissue at the centre of a large spot often disintegrates. On the surface, especially the undersurface, the conidiophores and conidia appear as a white powder over the decayed leaf tissue. The conidiophores arise from aggregations of hyphae which develop in the substomatal chambers of the host, grow up through the stomata and project from the leaf surface as brush-like clumps. The young conidiophore is a thick straight hypha, tapering slightly at its tip. The conidia are borne singly, though occasionally, in moist conditions, there is a short chain. They develop at the tip but soon become lateral, owing to the continued growth of the conidiophore, which itself becomes bent. The conidium is unicellular, elongate-oval, and white to pink. It germinates very rapidly; more than one germ tube may arise from one spore, the first-formed frequently developing from the pointed end of the spore. As Laibach (1921) observed, a germ tube may develop directly into a conidiophore.

All attempts to obtain a perfect state of *O. obliqua* were unsuccessful. It proved very difficult to collect overwintered *Rumex* leaves in their natural surroundings, for the fallen leaves disintegrate very rapidly. Some overwintered leaves were collected and examined in the spring, but no perfect state was found in any of the *O. obliqua* lesions. Similarly, when infected leaves were collected in autumn and overwintered in the laboratory, out of doors, or in a refrigerator for 3 months and then in the laboratory, no perfect state was formed in the lesions infected by *O. obliqua.*

The fungus was grown in agar culture and subjected to various conditions. The composition, concentration and pH of the medium were varied and so also was the circumambient temperature. *O. obliqua* grew and produced conidia on all the media used. Small, spherical, dark brown sclerotia subsequently developed on most media, but no perfect state appeared in any of the cultures.

Infection and growth of Venturia rumicis *in the host*

Infection was achieved when *Rumex* leaves were inoculated, either on the upper or on the lower surface, with an ascospore suspension of *V. rumicis*. When pieces of *Rumex* stem were inoculated with ascospores obtained from infected leaves, infection of the stem took place; a result which casts doubt on Grove's stem form of the species.

The ascospore, which may germinate very rapidly—within 8 hr. of

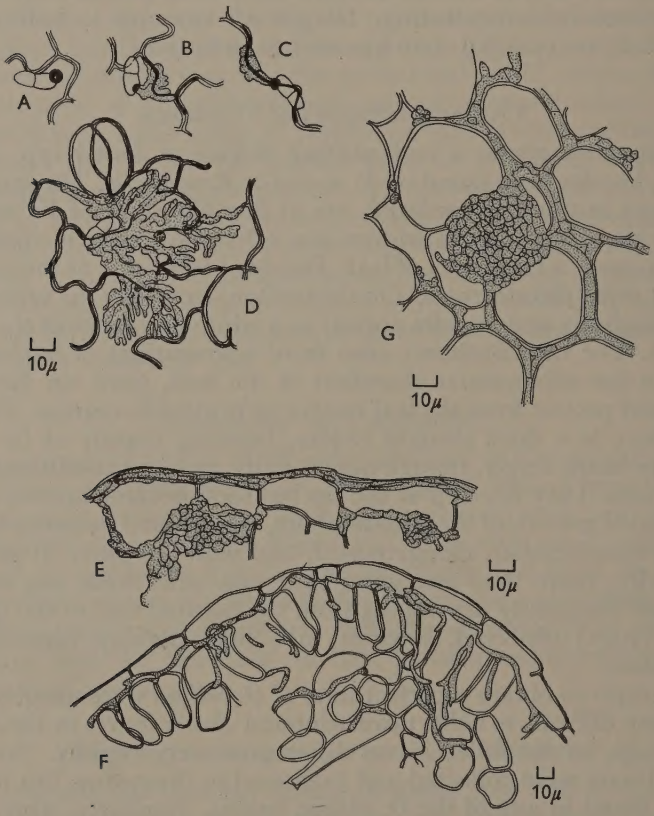

Fig. 1. *V. rumicis*. A–D epidermal strips of *Rumex* leaves showing stages in infection A. Ascospore germ tube has penetrated and 'alteration' of periclinal wall of epidermal cell is shown. B. A wide subcuticular (sc) hypha has developed. C. Ascospore germinated over junction of two epidermal cells. D. Extensive development of sc hyphae, radiating from the point of entry in a sheet-like layer. E. Part of a t.s. of a leaf, showing well-developed sc mycelium and stroma formation in the mesophyll. F. Part of t.s. of leaf at the edge of an infected spot; no sc mycelium, but se mycelium and intercellular hyphae in the mesophyll are shown. G. Part of a t.s. of a stem, showing intercellular hyphae in the cortex and a young intercellular fruit body. (Strips stained in cotton blue. 'Altered' areas in the periclinal walls of the epidermal cells in black; in the anticlinal walls indicated by the thick black line (middle lamella). Where subcuticular mycelium grows above the junction of epidermal cells, the epidermal walls are represented by a continuous ('altered') or dotted ('unaltered') line, instead of the usual three lines.)

liberation from the ascus, consists of two cells of unequal size (Fig. 1). When germinating on the surface of the host, usually only the larger cell produces a germ tube, but one germ tube may develop from each cell, and penetration of the host by two such germ tubes has been observed. The germ tube is short, seldom extending far beyond the spore before penetrating (Fig. 1 A–D).

The germ tube pierces the cuticle and a subcuticular mycelium develops which radiates from the point of entry, the hyphae forming an almost continuous sheet (Fig. 1 D). Though the individual epidermal cells are not penetrated, the epidermis does not prove an effective barrier, for hyphae from the subcuticular mycelium grow down between the cells and form a subepidermal mycelium, from which hyphae invade the other tissues of the host. While the germ tube does not appear to penetrate the epidermal cell walls, it seems to alter them in some way. When epidermal strip or cleared leaf preparations were stained with cotton blue, small greenish-blue areas were seen in the epidermal wall just below where the cuticle was pierced by the germ tube. These blue-stained areas are somewhat similar to the ones found by Corner (1935) when investigating penetration of epidermal cells by haustoria of powdery mildews. He found, even in some resistant hosts, that where the cuticle but not the wall of the epidermal cell was pierced by the haustorium, that an alteration in the epidermal cell wall could be detected by staining with cotton blue. By carrying out microchemical tests Corner was able to demonstrate that the cellulose of the cell wall was altered in the areas which stained blue with cotton blue. The nature of the change caused by the germ tubes of *V. rumicis* has not been demonstrated.

In many of the epidermal strip preparations the anticlinal walls of some epidermal cells also stained blue with cotton blue. The subcuticular hyphae grow above the junction of the epidermal cells (Fig. 1) and it is here that the staining of the anticlinal walls is noticed. The alteration in these walls possibly results from the growth of the fungus between the epidermal cells.

When the hyphae have passed through the epidermal layer they grow extensively immediately below the epidermis (Fig. 1E). The epidermal cells often become filled with tannin before they eventually break down. The subcuticular and subepidermal hyphae come together and form a stromatic layer below the cuticle.

In the other tissues the hyphae at first grow between the cells, causing little disorganization, but eventually the host cells become disorganized and the hyphae tend to come together and form a loose stroma, which is most apparent in regions where fruit bodies are developing.

At the edge of a leaf spot the hyphae are always in advance of the dead leaf tissue and appear not to be restricted in any way. In this region the leaf tissue is often greener than in the rest of the leaf.

The fruit bodies begin to develop between the host cells, but as they enlarge the host tissue round them breaks down and disintegrates. They often remain completely immersed in the tissues until they are mature. When young, the fruit bodies are spherical and closed, but as maturity is approached the upper part grows out as a papilla-like structure which penetrates and projects through the surface of the host. The papilla appears paler than the rest of the fruit body which is dark brown to black. When the fruit body is mature it becomes open to the exterior by an irregularly shaped pore, through which the ascospores are actively discharged.

The fruit bodies generally open to the upper surface of the leaf and this position of the fruit body is partly an effect of gravity. The fruit bodies are often separate, but frequently two or more coalesce to give a compound structure. These large fruit bodies may have one large ostiole, or two or three smaller ones, when mature.

Spine-like appendages may occur on the upper part of the fruit body, but are not always present. They are most frequently found on fruit bodies which are not deeply immersed in host tissue. When they are completely immersed it is only as maturity is reached that the upper part becomes free from host tissue, and this probably accounts for the frequent absence of spines on them. A spine is composed of a short non-septate hypha, which grows out from the surface of the fruit body, becomes thick walled, pointed and dark brown (Fig. 2F). Occasionally spines with one cross-wall have been seen.

Growth and development in culture

V. rumicis is homothallic. From a single ascospore, sown on a suitable agar medium, mature fruit bodies, producing viable ascospores, are obtained. The fungus grows slowly on all the common agar media. Its spread over the medium may be hastenened by the formation of new colonies which develop from spores discharged from fruit bodies formed in the initial colony. Mature fruit bodies in culture are very similar to those on the host. They invariably bear spine-like appendages.

V. rumicis can grow on a synthetic minimal medium lacking vitamins; it can utilize (*a*) sucrose and glucose as sources of carbon, (*b*) nitrate nitrogen, and (*c*) asparagine as the sole nitrogen source. It can tolerate a wide range of pH, but the optimum for both growth and reproduction is about pH 4. The optimum temperature for growth and reproduction is between 14 and 20° C. Reproduction is favoured by a low concentration of phosphate, a very small increase of which is inhibitory. This marked effect of phosphate shows that a phosphate–citric acid buffer is not a suitable buffer for adjusting pH when investigating the effect of pH on growth and reproduction.

Development and structure of the fruit body and centrum

The early stages of fruit-body development (Fig. 2A–C) in culture are made evident by the appearance of an initial, first recognized as a small hyphal knot (Fig. 2A); hyphae grow out from and round this knot and the initial soon becomes a dense and spherical aggregation of hyphae (Fig. 2B, C). Hyphal anastomoses are frequent in regions of fruit-body formation.

Sections are necessary to study further stages. At the small spherical stage there is little internal differentiation, an outer layer of thick-walled hyphae encloses a mass of deeply staining hyphae in the centre. Sections of older fruit bodies (Fig. 2 D, E) show differentiation of this central tissue; in the development of the centrum asci grow up in a layer into sterile tissue which is composed of vertically orientated pseudoparaphyses. Early in the differentiation of the centrum before asci are developed

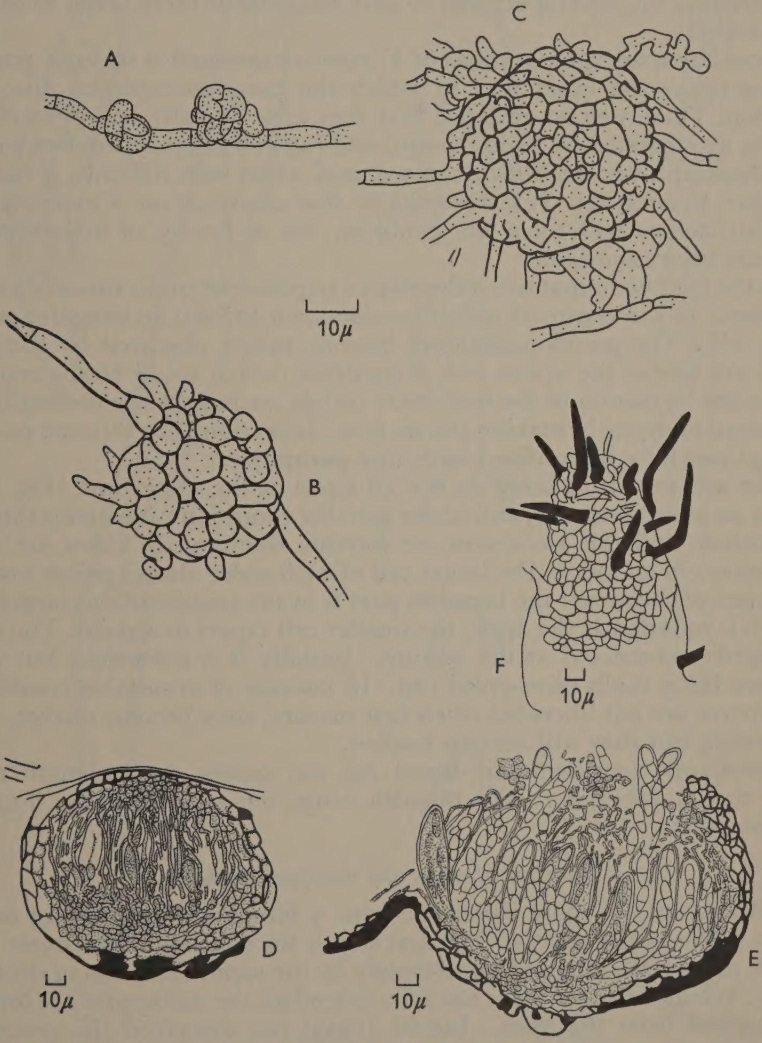

Fig. 2. *V. rumicis*. A–C. Stages in the development of the fruit body, as seen in agar culture. A. First indications of development. B. Fruit body initial a spherical aggregation of hyphae, outermost hyphae becoming thick walled. C. Initial larger than in B, and walls of outer hyphae thicker. D. L.s. young fruit body, shows asci growing up among pseudoparaphyses, apical attachment of pseudoparaphyses clearly shown. E. L.s. mature fruit body, some of the asci are extended; pseudoparaphyses somewhat obscured by the asci, and some appear free at their apical end. F. Apex of fruit body from agar culture, showing position and structure of spine-like appendages.

pseudoparaphyses are differentiated and are vertically orientated, thus showing that the vertical orientation of the pseudoparaphyses is not dependent on the upgrowth of the asci. Later (Fig. 2D) a layer of asci grows up among the pseudoparaphyses; the pseudoparaphyses are very evident, especially at the apex of the locule where their apical attachment can clearly be seen. The basal ends of the pseudoparaphyses are somewhat obscured by the developing asci so that attachment there is not so easy to demonstrate.

From the many preparations of *V. rumicis* examined it was not possible to determine the exact way in which the pseudoparaphyses arise and develop. There was no evidence that they arise apically and grow downwards, as suggested by Miller (1949) and Luttrell (1951 *a*), but free ends to pseudoparaphyses are difficult to see, and when seen difficult, if not impossible, to interpret. It seems unlikely that examination of more sections of fruit bodies will solve the problem, the difficulty of interpretation appears insurmountable.

As the fruit body matures it develops a papilla-like projection at its apex, the tissue in the centre of which breaks down to form an irregular ostiole (Fig. 2E). The pseudoparaphyses become rather obscured by the asci; some are free at the apical end, a condition which could be the result of either the formation of the fruit-body ostiole, or merely the cutting of the pseudoparaphyses in making the section. In sections like this one pseudoparaphyses may be confused with true paraphyses.

The asci in a fruit body do not all ripen at the same time (Fig. 2E); when an ascus is mature, and under suitable conditions, it extends through the ostiole and the ascospores are forcibly discharged. There are eight ascospores in an ascus. The larger cell of each spore always points towards the apex of the ascus; the broadest part is in the middle of this larger cell, which is rounded at the apex; the smaller cell tapers to a point. The spore is slightly constricted at the septum. Initially it is colourless, but when mature has a slight olive-green tint. If, because of unsuitable conditions, the spores are not liberated when first mature, they become darker, often brownish, but they still remain hyaline.

Ascospore measurements, based on 100 spores, were: length range 16·6–22·5 μ, average 19·5 μ; breadth range 6·6–9·9 μ, average 8·1 μ (at widest part).

Structure of ascus and ascospore discharge

All accounts of spore discharge from a bitunicate ascus agree on the main points of the process. The first step is the splitting of the outer layer of the ascus wall, followed immediately by the rapid extension of the inner layer. When the inner layer has fully extended, the ascospores are forcibly discharged from the apex. Ingold (1953) has described the process in *Sporormia intermedia*, referring to good accounts of the process in *Pleospora scirpicola* (Pringsheim, 1858) and in *Leptosphaeria acuta* (Hodgetts, 1917). In *S. intermedia* the inner wall extends until it bursts and the eight ascospores are discharged simultaneously. In *P. scirpicola* and *L. acuta* the spores are discharged successively from the tip of the extended ascus; in the former, the process takes place in water, but in *L. acuta* no active release of spores from the ascus occurs when the fruit bodies are immersed in water, but only in humid conditions.

Humid conditions are essential for ascospore discharge in *V. rumicis*. It

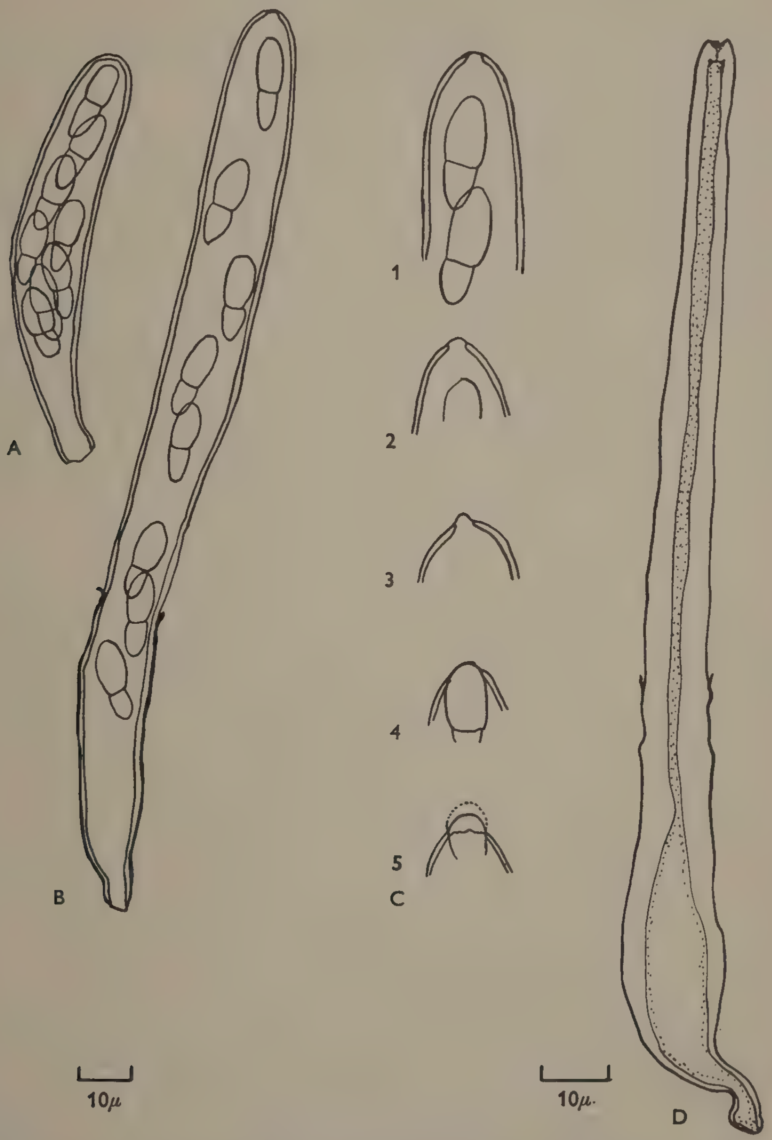

Fig. 3. *V. rumicis*. A–D. Asci mounted in water showing successive stages in release of ascospores. A. Mature ascus before extension. B. Extended ascus; outer layer of wall seen as a wrinkled sleeve at base of extended inner layer; ascospores in a row. C. Consecutive drawings of tip of extended ascus: 1–3, thin apical region protruding as a papilla; 4, ascospore in apical papilla; 5, indicates successive positions of the ascospore as it is forced through the papilla. D. Ascus from which all spores have been released.

can be watched under the microscope in comparatively normal conditions, when fruit bodies (either on the host or in culture) are kept in a glass ring moist chamber or when squashed fruit-bodies are mounted in water on a slide which enables more detail to be seen In a water mount the first indication of discharge is a slight overall enlargement of the ascus, with the ascospores seeming to become suspended in the middle and not touching the wall (Fig. 3A). Suddenly the outer layer of the ascus wall splits at the apex and the inner layer, surrounding the ascospores, extends smoothly but rapidly to form a long 'extension tube', about twice the original length of the ascus (Fig. 3B); the spores now lie in a row in the extension tube, the outer layer of the ascus wall remaining as a rather wrinkled sleeve at the base of the extended inner layer. The ascus may remain in this elongated condition for 2–3 min. before its spores are liberated, but more frequently liberation begins as soon as the ascus is fully extended. At the tip of the extension tube there is a thin area which protrudes slightly as a small papilla when the ascus is fully extended. When spore release is imminent the apical spore moves up so that its blunt end fits into the papilla (Fig. 3C). Pressure within the ascus gradually forces this spore through the papilla, which opens to form a pore. The spore is forced through until its broadest part is through and then it is violently ejected. The next spore immediately fills the apex of the ascus and this and following spores are released successively in a similar manner until all eight are liberated. Ejection of one spore and the replacement of it in the tip of the ascus by the next spore follows so rapidly that it is possible only to see the result. When a spore is ejected the ascus contracts slightly, but elongates again before the next spore is released; this retraction and elongation make the process appear jerky. The ascospores are always ejected singly; all eight may be discharged one immediately after the other, or there may be a time-lag between the release of each spore; again some may be released in quick succession followed by a delay before the rest are discharged. Immediately the eighth spore is released the extension tube of the ascus collapses and a considerable swelling of the inner layer of the ascus wall can now be seen (Fig. 3D). The apical pore is clearly visible. Occasionally asci are seen in which the outer layer of the wall has split, elsewhere than at the apex, but no release of spores from such asci has been witnessed. This abnormal splitting is probably caused by the rough treatment to which the asci are subjected when squashing the fruit body.

When viewing ascospore discharge from an unmutilated fruit body in a moist chamber, the only part of the ascus which can be seen is the extension tube, which slides out rapidly and either discharges its spores straight away or remains projecting for some minutes before doing so. At liberation the apical spore is forced part way through the ascus tip and then ejected with a jerk; the ascus contracts as this takes place and elongates again before the next discharge. The retraction and subsequent elongation are not quite equal, with the result that the extension tube becomes slightly shorter as the eight spores are released. Discharge from one ascus may be continuous or spasmodic; immediately the eighth spore is released the extension tube collapses completely, frequently falling back into the fruit-body cavity; it may collapse sideways and lie on the edge of the mouth of the fruit body. Some asci were observed to collapse before all their eight spores were discharged; this premature collapse often occurred when the humidity of the moist chamber had been allowed to fall. The ascus just sinks slowly sideways, there is not a rapid and complete collapse such as occurs at the termination of normal discharge. If moist conditions are restored these prematurely collapsed asci are able to extend again, but no release of spores from such re-extended asci has been seen.

More than one ascus may discharge ascospores from a fruit body at the same time; as many as five extension tubes have been seen protruding from one fruit body simultaneously.

The ascospores are shot a considerable distance, up to 1·5 cm.; this enables them either to land directly on another leaf, or to be carried away and dispersed in air currents. There is no periodicity to light, discharge taking place in continuous dark and in continuous light.

The account of spore discharge in *V. rumicis* agrees in most details with other accounts of discharge from bitunicate asci. It seems likely that the simultaneous discharge from several asci in one fruit body, which occurs in *V. rumicis*, is possible because the fruit body has no appreciable neck and opens by a rather wide ostiole. As Ingold (1953) pointed out, in fruit bodies with well-developed necks the neck often has a narrow canal, which permits the emergence of only one ascus at a time.

Structure of the ascus

Many accounts of the bitunicate ascus indicate that the wall at the apex of the ascus is thickened. For example, Higgins (1920, 1929) described the ascus wall of *Mycosphaerella bolleana* and of *M. personata* as 'apically thickened'; Wehmeyer (1946) considers an ascus with 'a much thickened wall at its tip' to be very characteristic of the genus *Mycosphaerella*; Müller & Arx (1950) and Menon (1956) state that in the Venturiaceae the ascus wall may be 'apically slightly thickened'.

The two-layered wall of the ascus of *V. rumicis* is of even thickness, except at the apex, where there is a small thinner area. It is only the inner layer of the wall which thins out at the apex, but since the outer layer develops an apical split at spore discharge, it presumably has a point of weakness at the tip. The outer layer appears to be fairly rigid and more or less inextensible, while the inner layer is capable of considerable extension and also of swelling.

When asci of *V. rumicis* are mounted in water or other liquids such as lactophenol, glycerine and saturated sucrose solution, the inner wall may swell. This does not occur in all asci, and has never been seen in very young ones. It is thought that asci which swell in this way have been damaged in some way on removal from the fruit body and mounting them. Asci of apparently similar age may behave differently. The swelling of the inner layer of the ascus wall is not confined to the upper part of the ascus, though it is perhaps most noticeable there. Prematurely elongated asci with a similar swelling of the inner wall layer are often seen in liquid mounts. The inner wall layer swells under normal conditions only at the liberation of the eighth spore, when the pressure in the ascus is released (Fig. 3D). It seems possible that the swelling of unelongated and prematurely elongated asci, found in liquid mounts, is the result of a similar release of tension in the ascus, but in this instance brought about abnormally. Factors which might contribute to such a release of tension could include plasmolysis, the external pressure supplied by the coverslip and damage to the ascus as a result of its abnormal treatment. Structural damage to the ascus, e.g. by sectioning or squashing, does allow the inner layer of the wall to swell.

In all the asci of *V. rumicis* in which a thickening was observed the phenomenon could be accounted for by a swelling of the inner layer of the wall. Thus, the ascus wall cannot be described as apically thickened—it has a thin region at the apex, but is otherwise equally thickened.

It is possible that the asci of other species which have bitunicate asci, described as apically thickened, may have the same structure as the ascus of *V. rumicis*—the thickened apex being the result of a swelling of the inner layer of the ascus wall. However, even if the bitunicate ascus cannot strictly be called apically thickened, the swelling of the inner layer of the wall is so characteristic that in the absence of mature and discharging asci, it may be used as a diagnostic character for the bitunicate ascus (Figs. 2, 4, 5).

DEVELOPMENT AND STRUCTURE OF THE CENTRUM OF FOUR RELATED LOCULOASCOMYCETES

Luttrell (1951*a*, 1955) postulated two main developmental types of centrum: the *Pleospora*-type in which the asci grow up in a layer from the base of the locule into persistent sterile tissue composed of vertically orientated pseudoparaphyses; and the *Dothidea*-type in which the asci arise in a more localized region in the base of the locule and grow up in a fascicle into pseudoparenchymatous, non-persistent sterile tissue.

To illustrate the *Pleospora*-type of centrum Luttrell used as his principal examples *Melanomma pulvis-pyrius* (Chesters, 1938), *Pseudotricha viridicoma* (Wehmeyer, 1941), and *Myiocopron smilacus*, *Ellisiodothis inquinans* and *Glonium stellatum* (Luttrell, 1944, 1948, 1953). All these papers include good descriptions of the development of the centrum. Luttrell considered that the descriptions of *Pleospora herbarum* given by Cavara & Mollica (1907) and Arnaud (1925) furnish good circumstantial evidence that this fungus also possesses a *Pleospora*-type of centrum. Some recent accounts of development in *Pleospora* (Wehmeyer, 1954, 1955; Moreau & Moreau, 1956) are somewhat conflicting and will be considered later.

Luttrell (1951*a*) also included *V. inaequalis*, basing his evidence on the work of Killian (1917) and Frey (1924), which though not conclusive gives a strong indication that *V. inaequalis* has the *Pleospora*-type. This view is supported by the work of Müller & Arx (1950), Arx (1952) and Menon (1956), which though not particularly concerned with centrum structure, described pseudoparaphyses in the fruit body of *V. inaequalis* and of other *Venturia* species.

For the *Dothidea*-type of centrum development Luttrell (1951*a*) used *D. collecta* (Luttrell, 1951*b*) as his principal example. The descriptions of

Mycosphaerella personata and *M. tulipifera* by Higgins (1929, 1936) and those of *M. cerasella* and *M. arachidicola* by Jenkins (1930, 1938) are among several cited by Luttrell to show that the *Dothidea*-type of centrum is

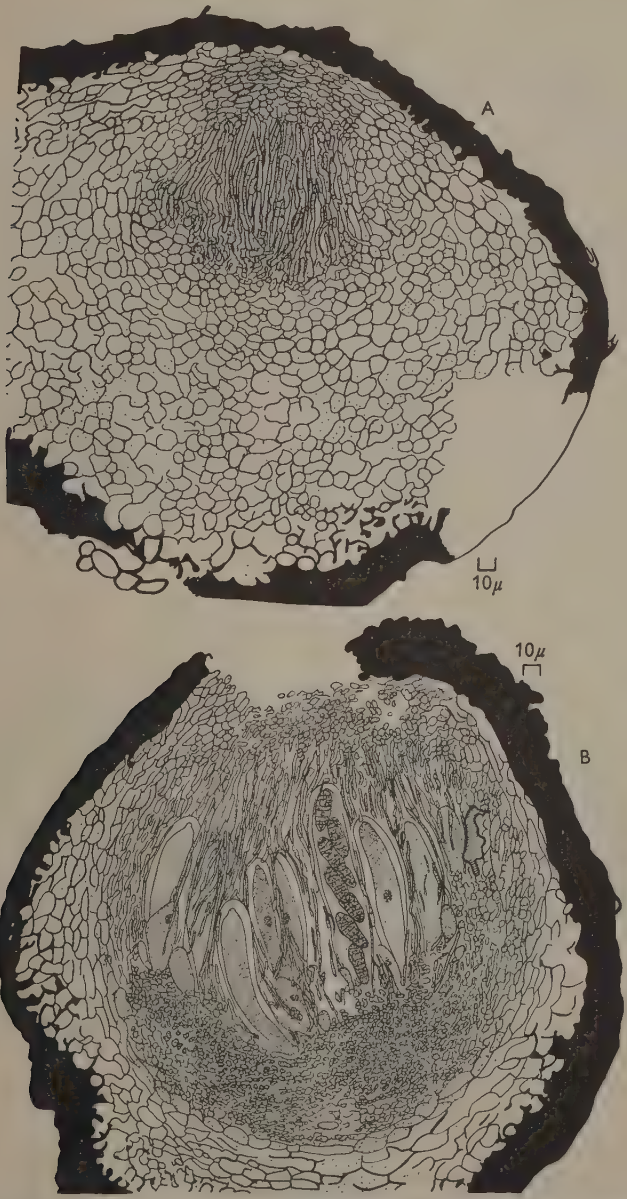

Fig. 4. *Pleospora herbarum.* Sections of fruit bodies showing stages in development. A. Young fruit body with vertically orientated pseudoparaphyses attached at both ends; asci not yet developed. B. Almost mature fruit body, showing a layer of bitunicate asci with pseudoparaphyses between them.

found in *Mycosphaerella*. These accounts are not primarily concerned with centrum development, but the descriptions and drawings are very clear and give ample evidence that the centrum is of the *Dothidea*-type

The account of centrum development in *V. rumicis* has shown that the bitunicate asci grow up in a layer from the base of the locule; in the locule the sterile tissue is composed of pseudoparaphyses which remain between the asci at maturity. This type of development indicates that *V. rumicis* is correctly placed in *Venturia* rather than in *Mycosphaerella*.

For comparison with *V. rumicis* the development and structure of the centrum of established species of *Venturia*, *V. inaequalis* and *V. pirina*, and also of *Mycosphaerella*, *M. maculiformis* (Pers.) Wint., were investigated. The centrum of *P. herbarum* was also studied because some variations in centrum development in *Pleospora* have been described (Wehmeyer, 1954, 1955; Moreau & Moreau, 1956).

PLEOSPORA HERBARUM

On malt extract agar the fruit body develops as a black, partly immersed, more or less spherical stroma: as it enlarges it becomes somewhat elongated and develops a short neck at the apex. At maturity the tissue at the centre of the neck breaks down. Compound fruit bodies may be formed, either by two or more of the simple ones becoming confluent, or by more than one locule developing in a single ascostroma, in which case, as Wehmeyer (1954) shows for *P. trichostoma*, the orientation of the separate locules is not necessarily similar.

Pleospora herbarum exhibits Luttrell's *Pleospora*-type of centrum development. The mature centrum (Fig. 4B) consists of a locule surrounded by a stroma and containing a basal layer of asci interspersed with pseudoparaphyses; the outer layers of the stroma are compacted and have become hard and dark coloured. The pseudoparaphyses arise before the asci (Fig. 4A); thus their vertical orientation is not dependent on the upgrowth of the asci. They are attached at both ends; in all preparations examined they appeared to be attached in this way. None which unequivocally showed naturally occurring free ends was observed. There was no evidence of radially ingrowing sterile filaments (Wehmeyer, 1954, 1955) in the centrum of any of the fruit bodies examined. While in some of the thicker sections the appearance of radial ingrowth was sometimes given, this effect is regarded as an artifact attributable to the cutting, or to the displacement of the pseudoparaphyses in preparing the sections, or both. The derangement of the pseudoparaphyses tends to be most marked in thick sections, where clearly there are more pseudoparaphyses to become multilated than there are in thinner sections.

VENTURIA INAEQUALIS AND *V. PIRINA*

The fruit bodies of these fungi develop immersed in the dead leaf tissue of the host. As they mature they project through the leaf surface, and when the asci are ripe the fruit-body cavity becomes open to the exterior by an irregularly shaped pore. The asci project through this pore when spore liberation takes place. The ascospores are two-celled and hyaline with an olive-green tint; they become dark olive-brown in old fruit bodies in which ascospore liberation has been delayed.

Longitudinal sections of fruit bodies of different ages showed that *V. inaequalis* and *V. pirina* possess a *Pleospora*-type of centrum, as described above. In sections of ripe fruit bodies the pseudoparaphyses are often somewhat obscured by the asci and ascospores; some of them also may appear to be free at their apical end, a phenomenon which is possibly the result of the breakdown of the apical tissue of the fruit body, in the

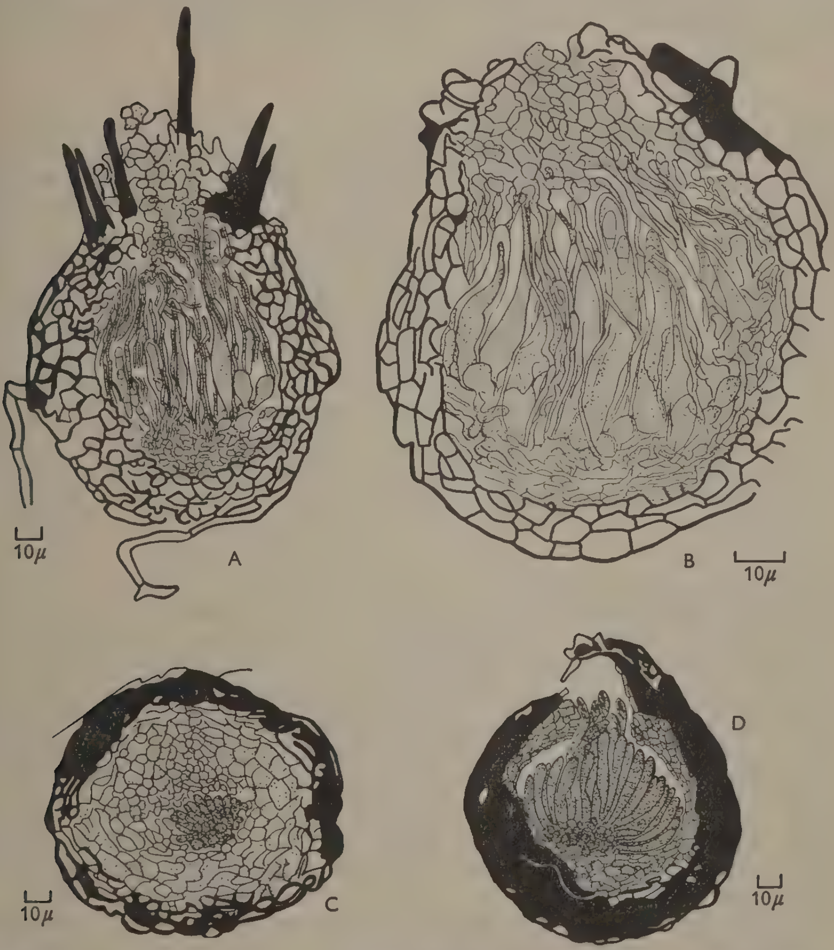

Fig. 5. A. *Venturia inaequalis*. L.s. of young fruit body showing apically attached pseudoparaphyses with asci growing up among them. B. *V. pirina*. L.s. of young fruit body showing a layer of young asci growing up among pseudoparaphyses which have no free ends. C. *Mycosphaerella maculiformis*. L.s. young fruit body; centrum consists of thin-walled pseudoparenchymatous sterile tissue with asci just beginning to develop. D. *M. maculiformis*. L.s. mature fruit body showing the fascicle of asci and absence of sterile tissue between the asci. (On oak.)

formation of the ostiole, but may also be caused by the cutting of the pseudoparaphyses in preparing the sections. In sections of young fruit bodies the pseudoparaphyses are more distinct, often clearly attached at both ends and with a layer of asci growing up among them (Fig. 5A, B).

Thus this investigation fully supports Luttrell's view that *Venturia* spp. possess a *Pleospora*-type of centrum and the genus is correctly placed in the Pleosporales.

MYCOSPHAERELLA MACULIFORMIS

There are two species of *Mycosphaerella* which are recorded as common on the overwintered leaves of broad-leaved trees; they are *M. maculiformis* and *M. punctiformis* (Pers.) Wint. (Winter, 1887; Munk, 1957). The arrangement of the fruit bodies and the size of the ascospores are used to separate the two species. The fruit bodies of *M. maculiformis* are aggregated, the aggregations appearing as small blackish patches on the leaf: the ascospores measure 9–14 μ × 2–4 μ. The fruit bodies of *M. punctiformis* are scattered and the smaller ascospores measure 7–8 μ × 2–4 μ. These differences are not very great, and as Winter (1887) and Klebahn (1918) have suggested, a thorough investigation of the two species is required.

Mycosphaerella sp. was found on overwintered leaves of beech, oak and horse and sweet chestnut. The fruit bodies were in groups and the ascospores measured 11–13 μ × 2–3 μ; hence it was tentatively accepted as *M. maculiformis.*

The fruit bodies developed immersed in dead leaf tissue, but projected from the surface at maturity.

Longitudinal sections of fruit bodies of different ages showed that the centrum possesses all the features of the *Dothidea*-type of development. At maturity (Fig. 5D) the centrum consists of a cavity filled with a fascicle of bitunicate asci with no sterile tissue between them. The cavity is surrounded by stromatic tissue, the outer layers of which are hard and dark coloured. The fasciculate arrangement of the asci was found in all sections of mature fruit bodies. Early in development (Fig. 5C) the sterile tissue of the centrum is composed of thin-walled parenchyma-like tissue in which the developing asci stain deeply; there are no pseudoparaphyses. As the asci grow upwards into the thin-walled tissue of the centrum it disintegrates.

The ascospores are two-celled and hyaline with a very slight greenish tint, thus very similar to newly matured ascospores of *V. rumicis*, *V. inaequalis* and *V. pirina*. In *M. maculiformis*, however, there is no subsequent darkening of the ascospores, such as occurs in these other three fungi.

Thus *M. maculiformis* affords another, previously undescribed, example of the *Dothidea*-type of centrum in *Mycosphaerella*, and it also provides additional support for Luttrell's view (1951 *a*, 1955) that the *Dothidea*-type of centrum is characteristic of this genus.

Munk (1953) questioned the use of the fasciculate arrangement of the asci as a taxonomic character, he considered it to be a 'negative character', a phenomenon resulting from the methods of examining the centrum, coupled with the rudimentary nature of the sterile tissue; since there is no interascicular tissue to support the asci, they become bunched when fruit bodies are mounted. The fascicle was very evident in *M. maculiformis* (Fig. 5) and when sections of wax-embedded fruit bodies were examined, before the wax was removed, the fasciculate arrangement of the asci was still distinct. In this fungus at any rate the fascicle appears to be real enough and it seems likely that this is true of Luttrell's examples (1951*a*, 1955).

DISCUSSION

In the classification of the Pyrenomycetes an apparently natural, as opposed to an artificial, system has been evolved during the last 20 years as more information about members of the group has accumulated. The erection of the subclass Loculoascomycetes by Luttrell (1955) seems a good attempt at a natural grouping. It is the logical consequence of the work of Nannfeldt (1932), Miller (1949) and Luttrell (1951*a*) and makes the delimitation of these ascostromatic Ascomycetes with bitunicate asci now generally acceptable.

Natural groupings within the Loculoascomycetes are much more difficult to define and there is as yet no general agreement as to how this group should be split up. Luttrell (1955) used characters of centrum development to make the primary divisions, while Müller & Arx (1950, 1954) placed more emphasis on external morphology.

In the examples used by Luttrell (1951*a*, 1955) to illustrate the *Pleospora*-type of centrum development there is no general agreement as to how the pseudoparaphyses arise, and variations in their development have been recorded. Since possession of pseudoparaphyses is the main difference between the *Pleospora*-type and the *Dothidea*-type, these variations should perhaps be investigated, for it may be that the *Pleospora*-type of centrum, as defined by Luttrell, might arise in more than one way, and if this is so it might be necessary to define the *Pleospora*-type of centrum more exactly before it is used as a criterion in classification.

Luttrell (1951*a*) and Miller (1949) contended that pseudoparaphyses arise from the apex of the young ascostroma as elongating hyphae with free ends, which grow downwards. These hyphae soon push down to the base of the locule where they become attached at their free ends. Further growth is by intercalary elongation.

In *Melanomma pulvis-pyrius* (Chesters, 1938) the pseudoparaphyses are formed by the lateral separation and subsequent elongation of hyphae in the centre of the young ascostroma. Their further growth is also by intercalary elongation. Thus the pseudoparaphyses never have free ends.

The divergent accounts of fruit-body development in *Pleospora* emphasize the difficulty of interpreting the course of centrum development, particularly the development and growth of the pseudoparaphyses. In the accounts of *P. herbarum* by Cavara & Mollica (1907) and Arnaud (1925) the exact origin of the pseudoparaphyses is not clearly explained. Wehmeyer (1955) described the development of pseudoparaphyses in *P. armeriae* and included it in a *P. herbarum* complex; it is separated from *P. herbarum* solely on ascospore size. In *P. armeriae* Wehmeyer found that the pseudoparaphyses arise as a somewhat irregular radial ingrowth of elongating hyphae, which soon become parallel in a vertical direction to form a palisade into which the asci grow up, and increase in length by intercalary growth. Wehmeyer considered that some, if not all, of the pseudoparaphyses remain free at one end. In the centrum of *P. trichostoma*, Wehmeyer (1954) described radially ingrowing hyphae, not attached at both ends, which branch and twine about each other. Only as the asci grow up do they become vertically orientated, being 'compressed into almost non-existent filaments by the enlarging asci, or even compacted into thick-walled pseudoparenchyma'. In the latter event the mature centrum would be like that found in the Pseudosphaeriaceae of the Dothideales, a similarity which suggests that comparisons of mature fruit bodies may be misleading. Moreau & Moreau (1956) concluded that centrum development in *P. herbarum* is similar to that described by Wehmeyer for *P. trichostoma*; however, they described the development of the pseudoparaphyses as hyphal filaments which grow down from the apex of the fruit-body cavity. If the figures and descriptions given by Wehmeyer (1954, 1955) and by Moreau & Moreau (1956) are compared, the difficulty of interpretation is apparent. For example, those of *P. herbarum* (Moreau & Moreau, 1956) and those of *P. trichostoma* (Wehmeyer, 1954) could equally well be used to illustrate Wehmeyer's description and interpretation of centrum development in *P. armeriae* (Wehmeyer, 1955).

It seems probable that a number of the variations recorded in the development of the *Pleospora*-type of centrum are largely due to differences in the interpretation of sections. A solution to this problem might be reached if the same worker investigated several fungi in which differences had been recorded. In this way recorded differences, which were solely the result of the difficulty of interpretation, might be avoided, and actual differences and similarities in development might be more easily recognized.

In the investigations of *V. rumicis*, *V. inaequalis*, *V. pirina* and *P. herbarum*, the similarity of the centrum development was demonstrated clearly, even though it was not found possible to determine the exact origin of the pseudoparaphyses. In none was there an indication that the pseudoparaphyses arose apically and grew downwards; they appeared more prominent at the apex of the locule, for at the base they were soon obscured by the developing asci. There was also no evidence that the pseudoparaphyses originated as radially ingrowing hyphae with permanently free ends; they appeared to be always attached at both ends. However, the number of pseudoparaphyses in the centrum appears to increase as the fruit body develops, and since it seems unlikely that an increase could be the result of the longitudinal splitting of the original pseudoparaphyses, it is difficult to visualize how it could arise without the development of some hyphae with free ends. The development of pseudoparaphyses was studied most extensively in *P. herbarum*, but even in thick longitudinal sections of the fruit bodies, free ends were not seen. The appearance of free ends may be simulated; the pseudoparaphyses are often cut when the fruit body is sectioned, which is almost inevitable with a spherical centrum; while in other preparations the pseudoparaphyses may become folded or otherwise displaced when the sections are mounted.

Such possibilities have to be considered when interpreting the various preparations.

The investigations of centrum development, in all four fungi, furnish additional support for the *Pleospora*-type of centrum (Luttrell, 1951 *a*, 1955). There is no indication that this type may develop in more than one way; support is given to the view that the variations described could be the result of differences in interpretation. The difficulty of interpretation makes it practically impossible to distinguish any other than clear cut, and therefore probably fundamental, differences in centrum development, and even though the exact origin of the pseudoparaphyses has not been ascertained, the present investigations have certainly shown that centra with pseudoparaphyses can be recognized as a distinct type; the evidence all suggests that Luttrell's *Pleospora*-type of centrum is a sound taxonomic criterion. Until more evidence is forthcoming, particularly from comparative developmental studies of fungi at present included in the Pleosporales, the *Pleospora*-type of centrum appears to be a good basis on which to erect the order.

In the Pleosporales Luttrell (1955) adopted, with certain reservations, the Venturiaceae as designated by Müller & Arx (1950). Müller & Arx included Venturiaceae in the Pseudosphaeriales, which is characterized by the possession of a spherical fruit body opening by a roundish pore. Ascospore characters are used in the subdivisions of the order; Venturiaceae has two-celled ascospores which are olive brown or greyish green when mature, while in the Mycosphaerellaceae the two-celled ascospores are colourless, even at maturity. Both these families at present include fungi with a pseudoparaphysate centrum and others with no pseudoparaphyses to the centrum. While Luttrell adopted Venturiaceae, based as it is on spore characters, he included in it only members of the Pleosporales. The Venturiaceae as emended by Luttrell includes therefore only those fungi with both olive tinted ascospores and the *Pleospora*-type of centrum.

Venturia and *Mycosphaerella* have certain features in common: besides the bitunicate ascus and ascostromatic fruit body, these include a simple unilocular fruit body and two-celled ascospores. In the classifications of Müller & Arx (1950) and Luttrell (1955) these common features are recognized, but it is in Luttrell's system only that the distinct and apparently fundamental difference in the development of the centrum in *Venturia* and *Mycosphaerella* is recognized; a difference which is clearly shown by the present investigations of the two genera.

For identification, the use of developmental criteria has obvious disadvantages; it is time-consuming and, certainly with herbariutm material not always possible. However, the mature state frequenely reflect, development, and as pointed out by Barr (1957) a complete devlopmentals study is not always necessary. For example, sections of the mature fruit body of *V. rumicis* (Fig. 2E) give sufficient information to show that centrum development is of the *Pleospora*-type, while those of *M. maculiformis* (Fig. 5D) show that it is of the *Dothidea*-type.

It may be argued that spore characters are much easier to use as criteria for identification than are developmental ones. The spore colour character, used by Müller & Arx to characterize Venturiaceae, was found to be a positive character for *V. inaequalis* and for *V. pirina*. However, in some newly ripened fruit bodies the colour of apparently mature ascospores was found to be not significantly different from that of ascospores of *M. maculiformis*, and it would be difficult with such specimens to separate *Venturia* and *Mycosphaerella* on this character alone. However, in herbarium material spore colour is a fairly reliable character; in *V. inaequalis* and in *V. pirina* a darkening of unreleased ascospores always took place after a fruit body was mature, while in *M. maculiformis* the ascospores never became similarly darkened.

While the two systems under consideration are generally adequate for the identification of *Venturia* and *Mycosphaerella*, the affinities of the genera appear to be shown much more clearly by the system of Luttrell than by that of Müller & Arx, and the evidence of the present work all suggests that in this respect Luttrell's system is also better for the Loculoascomycetes as a whole.

The present work has shown that the presence of spines on the fruit body of *V. rumicis* is an unreliable taxonomic character; for while spines were always present on fruit bodies produced in culture, they were lacking on many developed on the host. This frequent absence of spines on the host is possibly one of the main reasons why Winter's naming of the fungus was not universally adopted.

The centrum of *V. rumicis* has been shown to be of the *Pleospora*-type and not of the *Dothidea*-type, and the fungus is therefore correctly placed in the Pleosporales *sensu* Luttrell; its olive tinted two-celled ascospores refer it to the Venturiaceae, and its simple spherical fruit body to *Venturia*. *V. rumicis* is also correctly placed in the Pseudosphaeriales of Müller & Arx, by virtue of its spherical ascostromatic fruit body. In the Pseudosphaeriales, the colour of its two-celled ascospores is the factor which determines whether it should be assigned to *Mycosphaerella* of the Mycosphaerellaceae, or to *Venturia* in the Venturiaceae. In *V. rumicis*, as in the other species of *Venturia* and *Mycosphaerella* which were studied in the present work, spore colour may prove misleading. The mature ascospores of *V. rumicis* are initially very pale green; they may be discharged and germinate in this condition, but if their discharge is delayed they become a dark olive, characteristic of *Venturia*. Dark-coloured ascospores were not found so frequently in the fruit bodies of *V. rumicis* as in those of *V. inaequalis* and *V. pirina*, but this may possibly be explained by the differences in habitat and life cycle. Fruit-body production in *V. rumicis* occurs on the living host, and it is a fairly rapid process; in suitable conditions the ascospores may be discharged within 20 days of infection, and they are capable of germinating very quickly after discharge. On the other hand, the fruit bodies of *V. inaequalis* and *V. pirina* develop in the dead overwintering leaves of the host which are infected in the summer or autumn; they reach maturity in the spring, when release of acospores may often be delayed by unsuitable conditions for their discharge. No attempt was made to germinate the ascospores of these last two fungi.

As the ascospores of *V. rumicis* become darkened with age, the fungus may be correctly assigned to the Venturiaceae of Müller & Arx, and to *Venturia*.

Thus in the different classifications, with their different criteria for separating *Venturia* and *Mycosphaerella*, *V. rumicis* is shown to be a species of *Venturia*. The fungus is therefore correctly called *V. rumicis* (Desm.) Winter.

This work was carried out in the Botany Department of The Royal Holloway College, and I would like to thank all members of that Department for their encouragement; particularly I would like to convey my great debt to Mrs M. P. Topping, for her stimulating help and advice throughout the work. The work forms part of a thesis accepted for the degree of Ph.D in the University of London.

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DEIGHTONIELLA TORULOSA (SYD.) ELLIS AND *VERTICILLIUM THEOBROMAE* (TURC.) MASON & HUGHES ASSOCIATED WITH A TIP-ROT OF BANANA FRUITS

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(With Plate 31 and 5 Text-figures)

An outbreak of black-tip disease, caused by *Deightoniella torulosa*, in a plantation of Lacatan bananas was particularly severe where soil drainage was poor and atmospheric humidity high. Symptoms were more advanced than any recorded previously in Jamaica.

In a few specimens, there was extensive dry rotting of the inner pulp, from which *Verticillium theobromae* was isolated. *D. torulosa* was isolated from necrosed peel but not from decayed pulp. External symptoms included those of both black-tip and cigar-end diseases. The evidence suggests that this type of tip-rot is a result of primary infection by *D. torulosa*, followed later by *V. theobromae*.

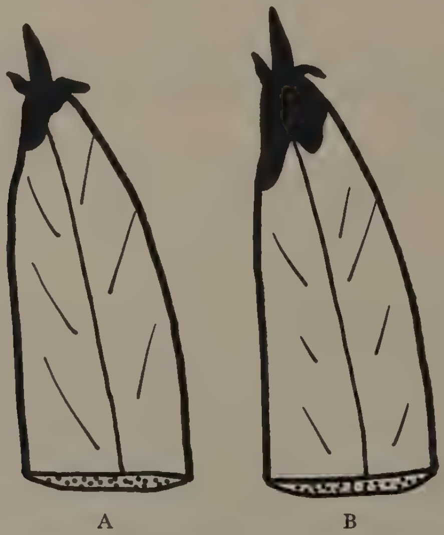
A finger-tip disease of banana fruits ('black-tip'), caused by *Deightoniella torulosa* (Syd.) Ellis, is of importance on the Cavendish variety in Bermuda (Ogilvie, 1928). Isolated examples of the disease have been found on the Cavendish and Lacatan varieties in Trinidad (Wardlaw, 1932) and on the Lacatan variety in Jamaica (Meredith, 1961*b*). Another finger-tip disease, popularly known as 'cigar-end', affects the Cavendish and other varieties growing in Panama, Trinidad, the Canary Islands, West Africa, Queensland and elsewhere (Wardlaw, 1935). In West Africa, two different fungi are associated with cigar-end: these are *Verticillium theobromae* (Turc.) Mason & Hughes (syn. *Stachylidium theobromae* Turconi) and *Trachysphaera fructigena* Tabor & Bunting (Anon. 1953, 1956, 1957*a*; Brun, 1954). Elsewhere, the disease appears to be caused by *V. theobromae* only. Recently, one isolated specimen of the Verticillium-type was recorded for the first time in Jamaica, on the Cavendish variety (Anon. 1957*b*). In this paper, an account is given of a finger-tip infection associated with both *D. torulosa* and *V. theobromae*.

DISEASE OUTBREAK

During September and October 1960, it was noticed on each harvest day that several bunches of fruit from a particular plantation were affected by a finger-tip infection. In severe cases, one or more fingers on every hand were diseased, while on others only a few fingers were affected (Pl. 31, fig. 1).

The plantation of Lacatan bananas is situated on the southern plain of Jamaica (St Catherine); the crop is a second ratoon with approximately 900 plants/acre. On the south-eastern border the plantation is protected by a row of tall wind-break plants. Under-tree irrigation, i.e. mechanical spraying of water, is carried out at fortnightly intervals during dry weather and less frequently during rainy spells. There was little weed present, but no attempt had been made to remove collapsed decaying banana leaves ('trash') from the plants. Nearby plantations (600 plants/acre) irrigated by flooding were also surveyed. Although isolated examples of black-tip disease were found, it was clear that the severe finger-tip infection described in this paper was rare, if not absent.

The most severe tip infection occurred on bunches of fruit from plants close to the wind-break. Here, the soil retained moisture longer than elsewhere; in addition, protection from wind resulted in slower drying out of foliage and fruit after rainfall, heavy dew formation or irrigation.



Text-fig. 1 A, B. Extent of black-tip infections on 20- to 30-day-old banana fruits.

Symptoms of disease and associated fungi

Symptoms were examined on fruit at different stages of maturity. The 'age' of fruit is expressed here as number of days from the time when the bunch shot (see Meredith, 1961*b*).

0- *to* 30-*day-old fruit.* Symptoms were identical with those of black-tip disease. A black discoloration of the peel was apparent just below the flower, but not extending for more than 1–2 cm. along the finger (Text-fig. 1 A, B). Diseased areas were irregular in outline and bordered by a narrow greyish-brown margin which sometimes had a water-soaked appearance. One side of the finger was often more affected than another. Infection was quite superficial, there being little or no invasion of the inner peel tissue or of the pulp (Text-fig. 2). Many conidiophores of *D. torulosa* were growing through the surface of necrosed peel, and the fungus was consistently isolated from these tissues. It was concluded that the disease was identical with black-tip, as originally described by Ogilvie (1928) and experimentally induced by Wardlaw (1932) and Meredith (1961*b*). No specimens of cigar-end disease were found, nor was *V. theobromae* associated with black-tip at this stage.

30- *to* 60-*day-old fruit*. Later stages of black-tip infection were evident. Blackening had spread along the fingers sometimes for distances exceeding 10 cm. (Text-fig. 3). Again, infection was restricted to the peel. *D. torulosa* was invariably present on the surface of, and in, the necrosed tissues. Cigar-end disease was not observed.

Harvested fruit (*over* 80 *days old*). In the majority of specimens examined, symptoms were typical of severe black-tip infection. In a few cases, however, additional symptoms not unlike those of cigar-end were present (Text-fig. 4 and Pl. 31, fig. 2). The region of decay commonly extended for more than 10 cm. along the finger. Towards the flower-end, the peel was black, shrunken and invariably ruptured in one or more places. Near the margin of the necrosed area, the peel was black and thrown into transverse folds. There was a sharp line of demarcation between healthy and diseased peel. An interesting distribution of fungi was observed. Distally, only conidiophores of *D. torulosa* were present on the peel surface. In the folded region, conidiophores and conidia of *V. theobromae* were numerous, forming an ashy or pinkish-grey coat; here, *D. torulosa* was uncommon.

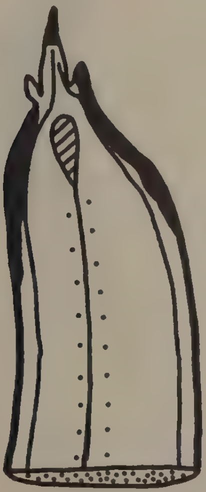


Fig. 2

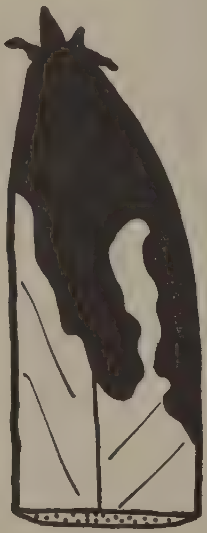


Fig. 3

Text-fig. 2. Diagrammatic section through 20- to 30-day-old banana showing superficial nature of black-tip infection.

Text-fig. 3. Extent of black-tip infection on 50-day-old banana fruit.

There was extensive internal decay, the pulp being rotted to a light-fawn-coloured, dry and fibrous condition towards the proximal end of the affected area (Text-fig. 5). Near the finger-tip, the pulp was dark coloured, crumbly and partially eaten away by various small insects. The rotted area was clearly marked off from adjacent healthy tissue. *V. theobromae* was readily isolated from the dry-rotted pulp and *D. torulosa* from some of the less decayed peel tissue.

It should be emphasized that extensive dry rotting of the pulp was not observed on specimens in which the peel was unruptured; in these cases, symptoms were those of black-tip only.

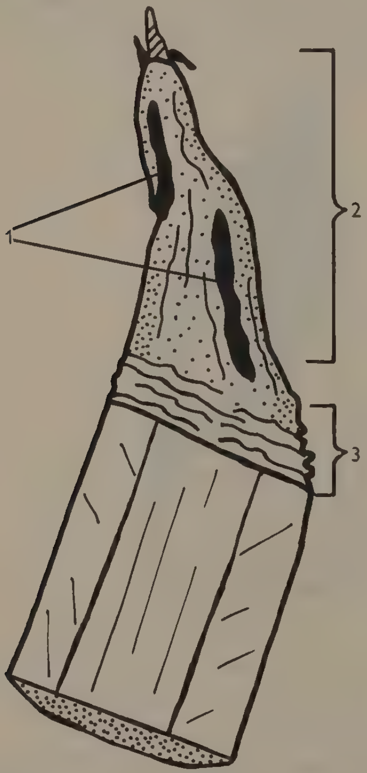


Fig. 4

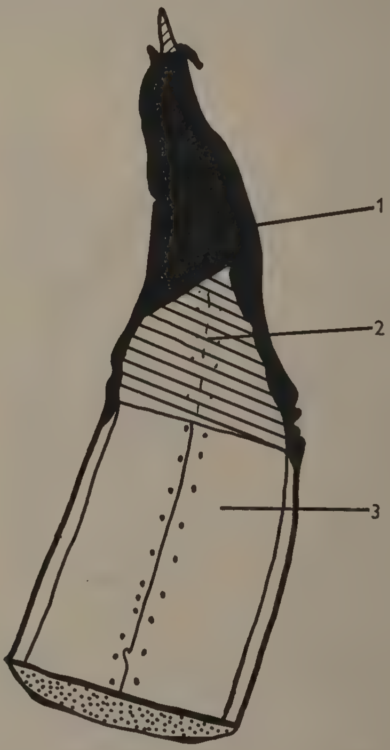


Fig. 5

Text-fig. 4. Banana tip infection associated with *D. torulosa* and *V. theobromae*. 1, Ruptures in peel; 2, region where conidiophores of *D. torulosa* were abundant on peel surface; 3, region where *V. theobromae* sporulated abundantly on peel surface.

Text-fig. 5. Longitudinal section through banana illustrated in Text-fig. 4. 1, Crumbly black pulp, partially eaten away; 2, fibrous, light coloured, dry-rotted pulp; 3, healthy pulp.

IDENTITY OF ASSOCIATED FUNGI

Deightoniella torulosa

All isolates of *D. torulosa* were identical with one (Herb. I.M.I., no. 80980) previously obtained from banana fruit-spot (Meredith, 1961*a*).

Verticillium theobromae

Conidiophores were usually solitary or in small groups on the surface of infected peel (Pl. 31, fig. 3). The conidia, borne at the ends of tapering phialides, were aggregated into rounded, mucilaginous, translucent heads. There was often fusion between individual spore heads. Measurements were as follows: conidiophores 150–400 × 4–6 μ; phialides 15–30 μ long; conidia 4–9 × 2–3·5 μ. These measurements, and the structure of the conidial apparatus, were very similar to those described by Turconi (1920), Wardlaw (1931) and Hughes (1951). An isolate of *V. theobromae* from an infected banana fruit has been deposited in the Herbarium of the Commonwealth Mycological Institute.

Discussion

The finger-tip infection described here, associated with *D. torulosa* and *V. theobromae*, does not appear to be identical with cigar-end disease as described by Wardlaw (1931, 1935). Also, it is clearly not a simple black-tip infection. The evidence suggests that the primary invader was *D. torulosa* and that *V. theobromae* followed at a later stage. Deighton (1933) suggested a similar succession in a tip-rot of Cavendish bananas in Sierra Leone. A possible sequence of events is as follows: initial infection by *D. torulosa* occurs through the perianth (Wardlaw, 1932), resulting in black-tip. As the finger becomes fuller, the necrosed peel ruptures. *V. theobromae*, either in the form of air-borne conidia or as mycelium from the decaying flower, then invades the exposed inner peel and pulp. In this way, some symptoms of both cigar-end and black-tip disease occur on the same finger.

V. theobromae is a common saprophyte on decaying leaves, bracts and other banana trash in the plantation (Meredith, unpublished). An explanation of why cigar-end rot is uncommon on the Lacatan banana in Jamaica must await further aetiological studies.

The distribution of black-tip inside the plantation suggests that its development is favoured by conditions of low evaporation. Wardlaw (1935) considered that close planting, poor soil drainage and low evaporation encouraged the disease; improved drainage and ventilation were suggested as possible control measures.

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EXPLANATION OF PLATE 31

Fig. 1. Harvested bunch of bananas, showing many fingers affected by tip-rot.
Fig. 2. Banana tip-rot associated with *D. torulosa* and *V. theobromae.*
Fig. 3. Conidiophores of *V. theobromae* growing out of infected banana-fruit peel.

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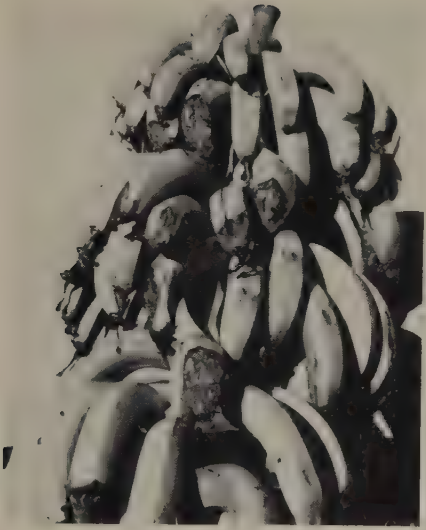

Fig. 1

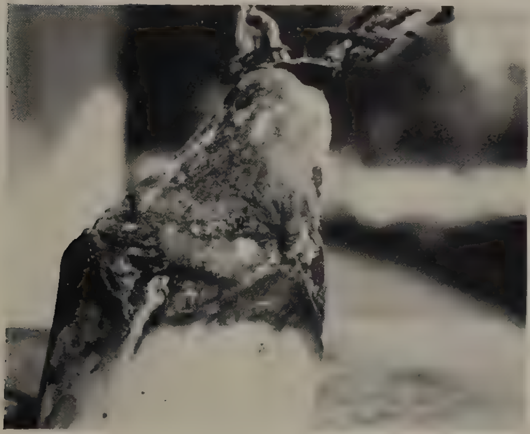

Fig. 2

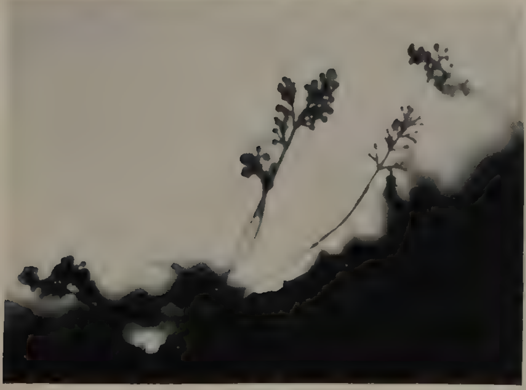

Fig. 3

(*Facing p.* 492)

Trans. Brit. mycol. Soc. **44** (4), 493–503 (1961).

HOST–PARASITE RELATIONS IN THE CHOKE DISEASE OF GRASSES

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(With Plates 32 and 33 and 3 Text-figures)

Grasses infected by *Epichloe typhina* showed no visible malformation of vegetative shoot apices, but after floral initiation the infected apex soon became malformed and hyphae grew out abundantly.

Experiments at different day lengths and temperatures showed that extensive growth of mycelium occurred only when the flowering apex was between the 'double-ridge' (~ 2 mm.) and 'spikelet-primordium' (~ 10 mm.) stages. If the apex grew very rapidly during this period, the 'choke' symptom did not develop, attack being limited to panicle branches or spikelets. If it grew slowly, the apex was killed when very small and a stroma did not form. Formation of a stroma required active but not particularly rapid growth of the apex during this vulnerable period. The significance of this behaviour is discussed in relation to the variation found in symptoms, and particularly to the production of 'blind' and 'partially choked' tillers.

Possible explanations are advanced for the vigorous growth of the fungus only in the very young inflorescences. This may be the only period during the ontogeny of the plant when sufficient meristematic cells remain in close juxtaposition for a sufficient time to allow the hyphae to absorb an adequate supply of nutrients.

Choke is widely distributed in Northern Europe, including Britain, where it often causes substantial reductions in the yield of grass seed. The causal fungus (*Epichloe typhina* (Pers. ex Fr.) Tul.) lives as a sparse fine mycelium in the above-ground parts of the plant; infection becomes evident at flowering when the inflorescence and two or three of the surrounding leaves become bound together by mycelium to form a stroma. In some species only the panicle or part of the panicle is invested in a stroma (Benedict, 1928; Sampson, 1933).

Little is known concerning the change from quiescence to rampant parasitism which occurs at flowering, or of the various degrees of expression of the disease in different species of grass host and in different years. The rapid development of the fungus may result from some internal stimulus in the host plant at flowering or simply from a favourable external environment which usually coincides with flowering. This paper describes observations and experiments on these questions. The plants used were clones of *Agrostis stolonifera* L., *Dactylis glomerata* L., *Holcus mollis* L., *Phleum pratense* L., and *Poa trivialis* L. They were established from diseased plants by taking only those culms which were bearing a stroma. These culms were placed in damp sand and the tillers which developed from the lower nodes were divided and grown on.

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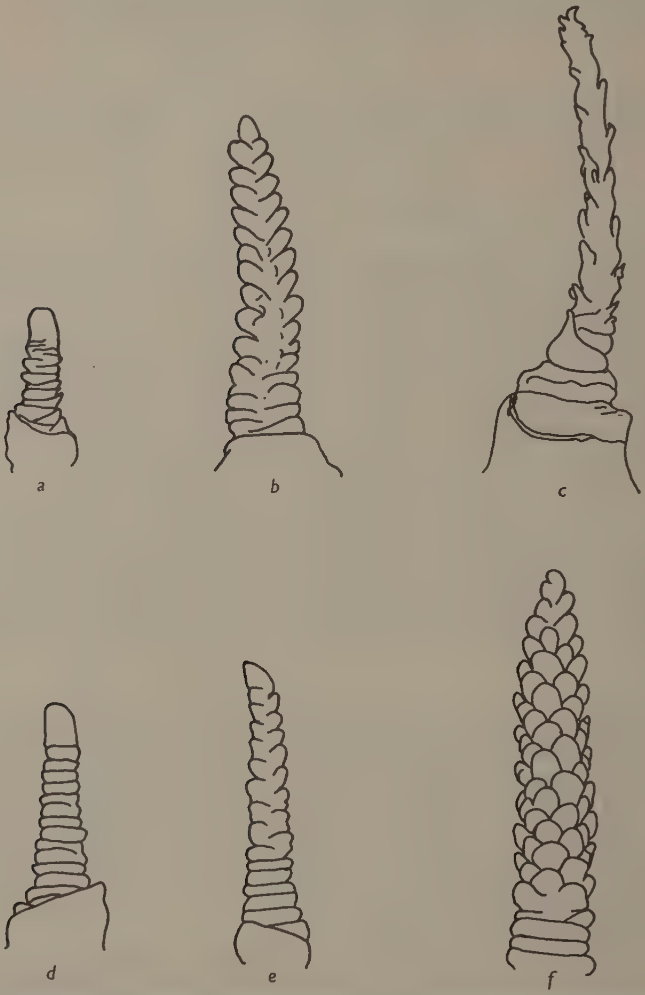
* Now at the School of Agriculture, University of Cambridge. The data presented in this paper were included in a thesis submitted to the University of Nottingham for the Ph.D. degree.



Text-fig. 1. Developing panicle of *Phleum pratense*. *a–c*, apices taken from plants infected with *Epichloe typhina*. *d–f*, apices taken from healthy plants. *a* and *d*, vegetative apices (about × 65); *b* and *e*, apices at double-ridge stage, *b* being slightly more advanced than *e* (× 50); *c* and *f*, apices at the panicle-branch stage of development, *c* being greatly malformed (× 25).

DEVELOPMENT OF THE PANICLE OF INFECTED GRASSES

Shoot apices of plants growing in pots outdoors were examined (Sharman, 1947) at regular intervals from early spring. In all species the vegetative shoot apex was normal when compared with similar apices of healthy plants or with published photographs. That of *P. pratense* was cylindrical and almost 1 mm. long (Text-fig. 1 *a*, *d*), that of *D. glomerata* distinctly compressed and in the other species it was cone-shaped. The change from the vegetative to the flowering phase was heralded by elongation and increase in girth. Protuberances formed in the middle region of the apex and extended in each direction to give the double-ridge stage (Text-fig. 1 *b*, *e*). Then, in healthy plants, the axillary branches grew out as the secondary and tertiary axes of the panicle branches (Text-fig. 1 *f*), but in infected plants the differentiation of the panicle branches was suppressed and elongation of the main axis continued, giving long, spindly and misshapen growths. The malformations were first seen when the apex was about 2 mm. long. In *H. mollis* and *P. pratense* the effect was usually severe, the diseased apex rarely grew longer than about 10 mm., and no recognizable parts were formed (Text-fig. 1 *c*; Pl. 32, figs. 1, 2). In *D. glomerata* the distal branches of the panicle sometimes developed until glumes were formed, the apex growing to 20–30 mm. before growth was arrested (Pl. 32, fig. 3). Shortly after these departures from normal development were first noted, fungal hyphae grew out from the apex and penetrated the young enclosing leaves, filled the spaces between the apex and leaf sheath and bound all together into a stroma. Elongation of the two or three internodes below the stroma carried it clear of the lower leaf sheaths.

Plants of *A. stolonifera* varied considerably in their pattern of development. In the most extreme examples, malformation was seen shortly after the double-ridge stage, as in the other species, and the pattern of stroma formation was similar. In contrast, the panicles of some infected plants grew normally. As differentiation progressed the upper florets developed more quickly than those at the base of the panicle. By the time the glumes and palea of the distal spikelets had developed fully, the glumes of the proximal spikelets were merely primordial ridges. Panicles of other plants of this species showed an intermediate type of behaviour; the leaves were not affected and the stroma was confined to the panicle only.

Other species in the field and some experimental plants showed partial choking of the panicles. These had some of the panicle branches invested in a stroma while other parts had escaped and bore apparently normal spikelets. The range of symptoms (Pl. 32, 33) suggests that there are marked differences in the rates of development of the inflorescence and of the fungus under different conditions. The stamens and pistils of such spikelets were usually found to be shrivelled and aborted. In one example, about thirty caryopses were found in a lightly affected panicle of *A. stolonifera*, but these were not viable. Sampson (1933) has described similar partially choked panicles occurring on plants of *Festuca rubra*.

RELATIONSHIPS BETWEEN THE AGGRESSIVE PHASE OF THE DISEASE AND FLOWERING OF THE HOST

Influence of time of flowering

To find whether the aggressive phase of the disease was attributable to internal changes associated with flowering or to the seasonal rise in temperature which occurs at flowering, plants were grown outside in pots and their times of flowering varied by manipulating day length and by applying growth substances. Plants were selected from a stock which had been maintained outside during the winter. On 14 March 1955, each plant was divided to give a clone of five propagants, each with two or three tillers. Nineteen clones of *P. pratense*, four of *H. mollis*, five of *D. glomerata*, and five of *A. stolonifera* were prepared.

Plants of each clone were distributed among five treatments: (*a*) kept in the normal environment, (*b*) kept in long days, (*c*) kept in short days, (*d*) treated with α-naphthaleneacetic acid, (*e*) treated with 2,3,5-triiodobenzoic acid. The long-day plants received supplementary tungsten light (*c.* 100–200 f.c. at the leaf surface) to give a total day length of 18 hr. The short-day plants were exposed to 8 hr. daylight only. Plants in treatments (*d*) and (*e*) were sprayed at weekly intervals, using an atomizer, with α-naphthaleneacetic acid at concentrations of 50, 100, 200 or 400 mg./l. or with 2,3,5-triiodobenzoic acid at 25, 50, 100 or 200 mg./l. All treatments were started on 27–30 March and continued until flowering or stroma exsertion was complete, except in the short-day treatments which were stopped on 23 June. Records were made of temperature, of the dates of flower initiation and of exsertion or panicle emergence, and of the number of 'choked' tillers expressed as a percentage of the total number of potential flowering tillers.

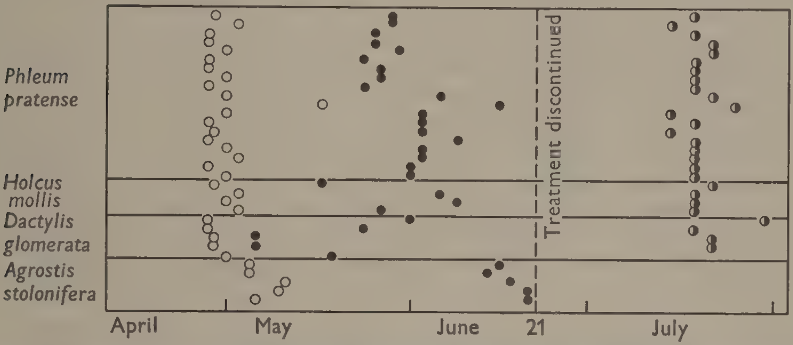
Table 1. *Percentages of potential flowering tillers of* Agrostis stolonifera *in the flowering treatments which bore normal panicles* (*N*), *showed partial stroma development* (*P*), *or were completely choked* (*C*). *Plants in short days did not flower*

	Normal environment			Long days			α-Naphthalene-acetic acid			2,3,5-tri-iodobenzoic acid		
Clone	N	P	C	N	P	C	N	P	C	N	P	C
A2	0	55	45	100	0	0	0	2	98	0	11	89
A4	0	0	100	100	0	0	0	0	100	0	45	55
A5	95	5	0	100	0	0	56	38	6	98	2	0
A6	90	10	0	100	0	0	100	0	0	100	0	0
A7	78	22	0	100	0	0	98	2	0	100	0	0

In the normal environment, plants of all species except *A. stolonifera* showed complete choking. Double ridges were first noted in *D. glomerata* on 20 April, and in *P. pratense* and *H. mollis* on 10 May (Text-fig. 2). Stromata were first exserted 12–15 days later and within 3 weeks all tillers were choked. Flower initiation was first seen in *A. stolonifera* on 27 April. There was considerable variation in the amount of choking among the five clones of this species (Table 1), ranging from plants with only fully choked tillers to plants with some apparently unaffected panicles. Further observations on these panicles showed that very few florets reached anthesis and no caryopses were found when the panicles were finally harvested.

In long days, plants of all species initiated flower primordia by 16 April (Text-fig. 2). All plants of *P. pratense*, *D. glomerata* and *H. mollis* were completely choked. All panicles of *A. stolonifera* were quite free of stromata but the florets contained only shrivelled pistils and stamens. Large amounts of mycelium were found in the glumes, palea, ovaries and anthers.

In short days, until 21 June, the plants were prostrate, all apices were vegetative and there was no sign of any damage by the fungus. They were then subjected to the natural day length of 18 hr. Within about 4 weeks all species except *A. stolonifera* had produced stromata. None of the five clones of *A. stolonifera* produced either panicles or stromata; their apices were examined on either 28 July or 17 August and were found to be vegetative.



Text-fig. 2. Dates when stroma or panicle emergence were first noted in clones of four species grown under long days (white circles), normal environment (black circles) or in normal environment after transfer from short days on 21 June. (Plants of each clone are shown on the same horizontal line.)

Table 2. *Temperature (° C.) during two parts of the experimental period*

	During the period 20–30 April			From flower initiation to stroma exsertion		
	Mean minimum	Mean maximum	Mean	Mean minimum	Mean maximum	Mean
Normal environment	5·5	17·5	11·5	6·5	16·0	11·0
Long day	9·5	23·0	16·5	9·0	23·0	16·0
Short day	7·5	18·0	13·0	12·0	24·0	18·0

The dates of flowering of plants treated with α-naphthaleneacetic acid or with 2,3,5-triiodobenzoic acid were not materially different from those kept in the normal environment. No differences in the external morphology of the shoot apex during development of choke or in the general appearance of the plants were noticed, although the treated plants were less vigorous.

This experiment showed that differentiation of the inflorescence was an essential prerequisite for the active development of mycelium and for stroma formation, which then proceeded over a wide range of temperatures (Table 2). Except in *A. stolonifera*, advancing or retarding the date of flower initiation had no effect on the degree of choking.

The mean temperature during the period when flowers are normally initiated (the early part of the experiment) was higher in the short-day than in the normal environment, but was not as high as in the long-day treatment. However, the short-day plants were exposed to moderate temperatures for a prolonged period. The failure of plants of *A. stolonifera* to flower when subsequently transferred to long days may have resulted from devernalization during this short-day period.

Influence of temperature and light intensity

The influence of temperature on development of the inflorescence in infected plants of *D. glomerata* (3 clones), *H. mollis* (2), *P. pratense* (3), *A. stolonifera* (2) and *P. trivialis* (1) was studied in the plant growth rooms described in Hudson (1957, p. 184). The material was obtained by splitting up large plants, which had overwintered outside, into eight uniform pieces, and potting these in compost on 12 March. The plants were kept in a 10 hr. photoperiod until 29 March, and two plants of each clone were then transferred to the following treatments, each with an 18 hr. photoperiod: (*a*) temperature 25° C., light intensity 1300 f.c. at the leaf surface; (*b*) temperature 25° C., light intensity 450 f.c.; (*c*) temperature 10° C., light intensity 1200 f.c.; (*d*) temperature 10° C., light intensity 400 f.c. The plants were inspected daily and date of emergence of each panicle or stroma was recorded. After 6 weeks, and again after 8 weeks, one plant of each clone was removed and the apices of all tillers examined.

There were no consistent differences between plants kept at the different temperatures or light levels, variation within being greater than that between treatments. The majority of plants in all treatments initiated flowers and produced normal stromata. However, all plants of one clone of *D. glomerata*, one plant of *H. mollis*, six plants of one clone of *P. pratense*, and one clone of *A. stolonifera* remained vegetative. The second clone of *A. stolonifera* produced panicles without any visible disease symptoms. The plants of *P. trivialis* produced a mass of weak tillers, many of which died either before or after producing stromata. One tiller of *P. pratense* was peculiar; three lower nodes of the apex had developed to the spikelet-bud stage but the distal part was purely vegetative (Pl. 32, fig. 5). A number of culms of *H. mollis* and *P. pratense* terminated in a typical flag leaf; in these the apex was malformed and invested with mycelium. In some tillers the apex had emerged from the leaf sheath (Pl. 32, fig. 6). This symptom was also found in plants growing naturally in the field.

The experiment was repeated with one clone each of *D. glomerata*, *P. pratense* and *A. stolonifera*. From periodic dissections the lengths of the apex and internodes of the main tillers, the vegetative or floral nature of the apex and the degree of choking were recorded. After overwintering out-of-doors, the plants were kept from early spring in a room at 2° C. in diffuse light. They were removed on 15 June, divided, and potted in compost. After a short time in a 10 hr. photoperiod they were distributed among the following treatments, all with an 18 hr. photoperiod: (*a*) temperature 10° C., light intensity 950 f.c. at the leaf surface from fluorescent tubes only; (*b*) temperature 10° C., light intensity 1100 f.c. from fluorescent tubes supplemented with tungsten filament bulbs; (*c*) temperature 27° C., light intensity 1000 f.c., fluorescent only; (*d*) temperature 27° C., light intensity 1100 f.c., fluorescent supplemented with tungsten light. A further treatment (*e*), in which plants were grown outside under normal day lengths and temperature, was included.

Floral initiation and further development of plants in the controlled environments were slower and more variable than in the plants grown outside. The addition of tungsten to fluorescent light did not make any appreciable difference. No plants of *A. stolonifera* initiated flowers.

All plants of *D. glomerata* examined after 11 days had flower initials, those grown outside being the most advanced. Many more tillers produced flower initials in (*a*), (*b*) and (*e*) than at the high temperatures. The growth of the developing panicle was stopped by stroma formation at the stage when spikelets were distinguishable on the upper part of the panicle, which was then about 20–40 mm. long. Vegetative tillers did not elongate. Tillers which initiated flowers elongated normally, starting when the apex was 1·5–2·0 mm. long, i.e. when panicle-branch primordia were just visible. The buds in the leaf axils of the first and second elongated internodes had developed some leaves, and the apices of some had formed panicle-branch primordia which were malformed (Pl. 32, fig. 4). Others were still enclosed in the prophyll and were at the double-ridge stage.

Very few plants of *P. pratense* in treatments (*a*) and (*b*) initiated flowers, and only two plants produced normal stromata. In some plants atypical apices, similar to those described previously, were found (Pl. 32, fig. 5). In treatments (*c*) and (*d*) some plants in which the panicle had developed to a length of 7 mm. produced typical stromata. The symptom described previously (Pl. 32, fig. 6) also occurred. In treatment (*e*) only typical stromata were produced, the developing panicle attaining a length of 10–14 mm. before its growth was arrested. In all treatments there was considerable elongation of the vegetative tillers.

In these experiments flower initiation and subsequent choking were disappointingly poor. This was certainly due in part to the way the material was prepared, as the tillers must have been at different stages of growth and in different physiological condition. Another factor was insufficient knowledge of the physiology of flowering to ensure that the grasses always flowered at a predetermined time.

Influence of transference to short days

Clonal material of *D. glomerata*, overwintered out-of-doors in pots, was transferred to an 8 hr. photoperiod at intervals during the period when flowers were being initiated. Most plants produced normal stromata, but some abnormal stromata were found. In these, that portion of the sheath of the outermost leaf covered with mycelium was 7–50 mm. above, rather than immediately above, the node. On some culms the stroma did not encircle the sheath completely. The stromata were smaller than usual, extending over 15–25 mm. and involving only two leaves. The apices were only 4–9 mm. long with panicle branches and spikelet primordia, and were completely invested with mycelium. Internodes below each stroma had elongated normally.

It has been noted that transfer of grasses to short days after flower initiation interferes with normal panicle development. For example, Wycherley (1952) transferred plants of *D. glomerata* to short days after 5 and 15 days in continuous light. Although panicle development proceeded to the stage of recognizable stamens and ovules, the panicles never completely emerged but remained within the ultimate leaf. In the present study, it appeared that floral initiation in infected plants was followed by the usual rampant growth of the fungus, which spread from the apex to the young leaves. On transfer to short days, the growth of the panicle appeared to have been slowed down and the mycelium, after investing the apex, had not been able to continue growth through the leaves. Continued growth of the basal meristem had produced a portion above the internodes free of stroma. Because of the slower growth of the fungus, fewer leaves were involved in the stroma.

Discussion

In the experiments just described, plants of five species were subjected to different environments. Only under those conditions which led to the host forming flower initials was the systemic fungus incited to grow out from the stem apex. The development of the panicle departed from its normal pattern at the same time as the activity of the fungus increased. The formation of the secondary meristems which give rise to the various parts of the panicle was arrested, while elongation of the culm proceeded unchecked. Simultaneously, the fungus formed a compact stroma. This held the panicle so firmly that for mechanical reasons alone further development was impossible. The association of vigorous growth of the fungus with floral initiation can be separated from any seasonal factor acting directly on the fungus; it must be due to some change which occurs in the shoot apex after floral initiation.

The disease may develop in one of three main directions:

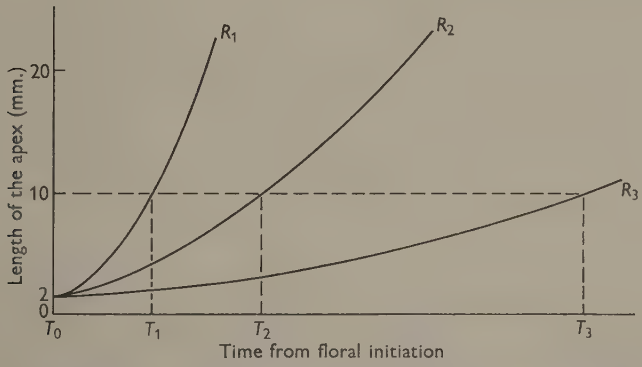
(*a*) The apex can differentiate and develop normally. Normal panicles with healthy seed have been reported by Sampson (1933). This appears to be always so with the strains of *Festuca pratensis* in New Zealand which, although containing an endophyte strongly resembling *E. typhina*, never produce stromata (Neill, 1941). In the experiments described above, panicles were produced but no viable seed was collected.

(*b*) In the typical diseased plant the panicle may develop to a certain extent, but eventually the fungus binds it into a stroma and further growth is prevented.

(*c*) The apex may be killed at a very early stage and the mycelium does not penetrate the surrounding leaves.

These different courses of the disease may be explained by assuming that (i) the conditions in the host favour the rapid development of the parasite for only a limited period, (ii) the rate of development of the host panicle is influenced by the environment, (iii) the growth of the fungus is less affected by the external environment than is the development of the panicle. The evidence from these experiments suggests that the phase of rapid growth of the fungus commences soon after the apex has developed to the double-ridge stage (2–10 mm. long). The stroma is fully developed by the time the apex is 20–40 mm. long. The stroma never develops unless the fungus is growing rapidly by the time the panicle has developed to the spikelet-primordium stage (about 8 mm. long). As a finite period of time must be required for development of the stroma, the rate at which the flowering apex passes through this vulnerable stage could well explain the failure or success of the host to escape from the formation of a stroma.

It may be assumed that in different environments the apex will grow at different rates and we may consider those that are either relatively rapid (R_1), intermediate (R_2), or slow (R_3) (Text-fig. 3). The period of time (T_1, T_2, T_3) during which the apex is in the vulnerable condition, i.e. when it is 2–10 mm. long, will depend on the rate of development. If the growth of the apex is rapid the fungus will have made little growth by the time the vulnerable period is over. Under these circumstances a normal panicle will result. If growth is checked then the panicle may be partially affected. If the growth of the apex is less rapid then the fungus will make much growth and form a stroma. Under conditions of very slow growth the fungus will overwhelm the apex and its potential supply of nutrients will be exhausted before it can form a stroma.



Text-fig. 3. Hypothetical curves for the growth rate of developing panicles under different environments. Assuming the apex is vulnerable to fungal attack when it is between 2 and 10 mm. long, then T_1, T_2 and T_3 show the times taken to pass through this stage with different growth rates of the apex (R_1, R_2 and R_3).

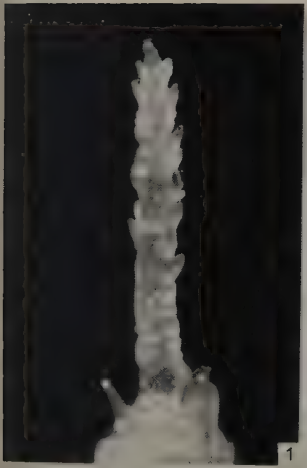
There is a good deal of evidence which suggests that both the rate and pattern of development of the flowering apex is considerably influenced by environmental factors such as day length (Cooper, 1952; Gott, Gregory & Purvis, 1955; Wycherly, 1952) and temperature (Cooper, 1958). Differences in the early growth of the inflorescence in any one species are therefore likely, and could account for most of the variation in stroma formation between different years. Differences between species are likely to arise from inherent differences in the rates at which the inflorescence passes through the vulnerable state.

The changes which occur in the apex when it passes from the vegetative to the flowering state, and which lead to such rapid growth of the fungus, are unknown. As *E. typhina* grows readily on a wide range of culture media, it is unlikely that it requires specific substances that are present only in the tissues of the young differentiating inflorescence. Inhibition of the fungus in vegetative tissues, due directly to one or more chemical substances, seems equally unlikely. Its ready development on dead leaves suggests that it is inhibited in living elongated tissues simply because of the lack of nutrients. It is unable to penetrate cells and must rely on the very limited substrates available in the intercellular spaces; these would be substantially increased following death of the cells.

Meristems would appear to be the only regions where the mycelium, because of its intercellular location, could establish sufficient contact with cytoplasm to obtain an appreciable supply of substrate. In the vegetative apex elongation proceeds rapidly, the hyphae not remaining in contact with any group of meristematic cells for a sufficient length of time to allow luxuriant local growth. The young flowering apex, however, has a much larger volume of meristematic tissue and the cells elongate less rapidly and are closer together for a much longer period than in the vegetative apex. It is conceivable that this morphological juxtaposition of meristematic tissue allows the local development of the hyphae, these perhaps growing no more rapidly at first than in vegetative apices but the growth proceeding within the same tissue for a longer time with an ample supply of substrate. Increased localized growth could in turn lead to death of some host cells, perhaps due to depletion of the supply of oxygen, and thus the supply of nutrients to the hyphae would be enhanced. One grave objection to this simple speculation is that luxuriant growth of the fungus would be expected in other regions of non-elongating meristematic tissue as, for example, in dormant buds. Such has never been noted.

Stimulation of the fungus by qualitative or quantitative changes of growth substances in the host is a less satisfactory explanation. There is little evidence that fungi respond to auxins and gibberellins, or on the nature of the changes in concentration of these substances during the early growth of the apex. Other substances are more likely to be concerned. *E. typhina* is known to be heterotrophic with respect to thiamin and possibly for certain amino acids (Lilly & Barnett, 1949), and changes in concentration of these or similar substances might be involved.

However, in the vegetative apex (Sunderland, Heyes & Brown, 1956) the outer layer or tunica, consisting of the superficial layer of small cells of the apical meristem and the young leaf primordia, shows very low metabolic activity. The cells of the tunica have a lower protein content and a lower respiration rate per unit protein, but a higher protein concentration, than have the larger underlying cells of the corpus. In the floral apex it is probable that the flower primordia are also regions of high protein concentration and low metabolic activity, but of much greater volume and persisting in this state for a longer time, than in the leaf primordia. It is likely that the rapid growth of the mycelium of *E. typhina* commences in tissue very comparable to that of the tunica. Although these tissues will eventually give rise to different organs and large differences in composition would be expected during expansion, such changes would be expected at a later stage of differentiation than that at which the fungus is stimulated. However, until analyses of the outer layers of the flowering apex have been made and until some knowledge of its physical and chemical differences



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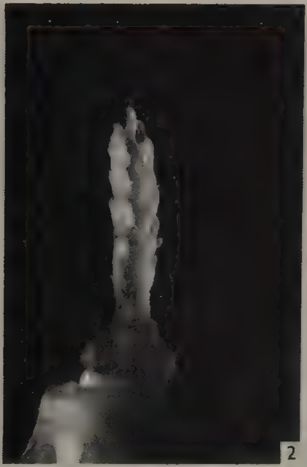

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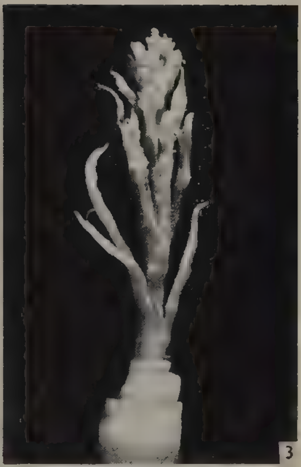

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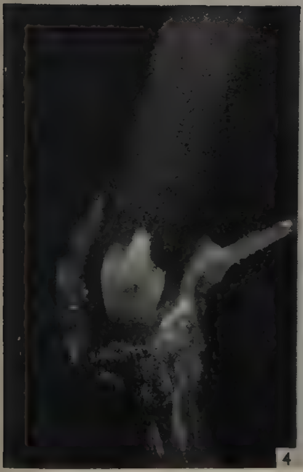

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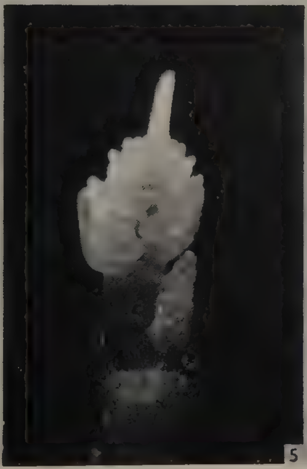

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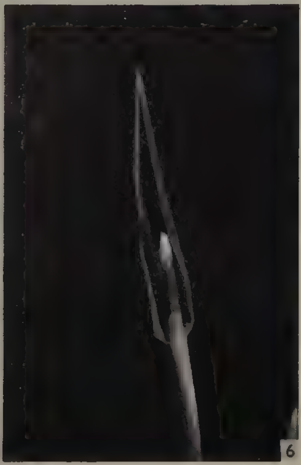

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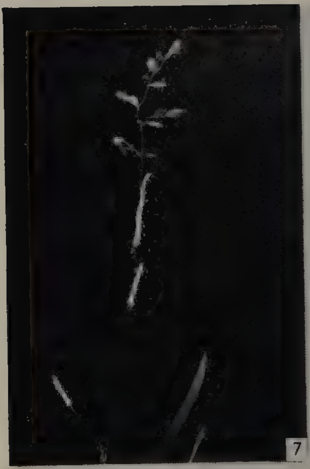
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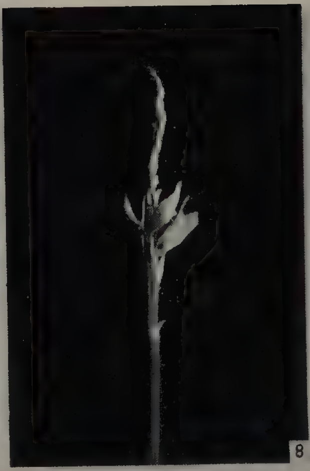
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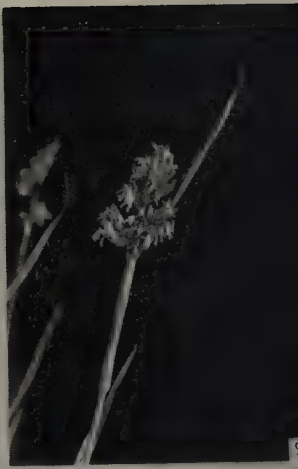

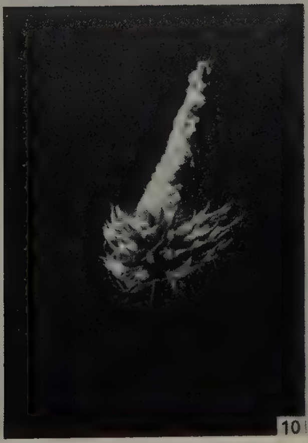
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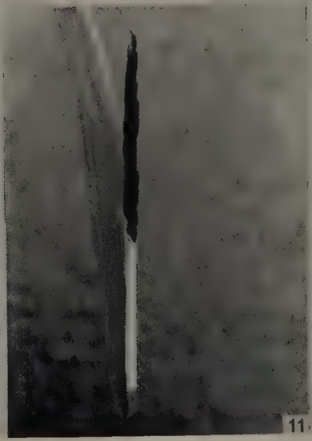
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from the vegetative apex is available, no adequate explanation of the stimulating mechanism is possible.

I wish to thank Prof. F. L. Milthorpe for his interest and advice during the progress of this study and in the preparation of this paper.

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EXPLANATION OF PLATES 32 AND 33

Plate 32

Fig. 1. Flowering apex (about 4 mm. long) of a diseased plant of *Phleum pratense*.

Fig. 2. A similar apex (about 3 mm. long) of a diseased plant of *Holcus mollis*.

Fig. 3. A diseased panicle of *Dactylis glomerata* (about 20 mm. long) taken from a developing stroma. The lower panicle branches are much malformed; the upper ones show recognizable spikelets.

Fig. 4. The apex of a bud (about 2 mm. long) in a leaf axil on a diseased culm of *Dactylis glomerata*. It has developed past the double-ridge stage and shows malformation.

Fig. 5. The stem apex (about 3 mm. long) of a plant of *Phleum pratense*. Some nodes have initiated flowers, while the upper part is vegetative.

Fig. 6. Culm of *Phleum pratense* in which the apex has been killed at an early stage.

Plate 33

Partially affected panicles bearing stromata of *Epichloe typhina*. Fig. 7, *Poa trivialis*; Fig. 8, *Holcus mollis*; Fig. 9, *Dactylis glomerata*; Fig. 10, *Phleum pratense*; Fig. 11, *Agrostis stolonifera*.

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A COMPARISON BETWEEN *OPHIOBOLUS GRAMINIS* AND *OPHIOBOLUS GRAMINIS* VAR. *AVENAE*

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Six isolates of *Ophiobolus* from oat crops in mid-Wales were compared with an isolate of *O. graminis* from wheat. The isolates differed in cultural appearance and in pathogenicity to various cereals, but five isolates were considered to be *O. graminis* var. *avenae* on the basis of ascospore measurements. The sixth isolate agreed closely with *O. graminis*, and is believed to be the first record of the short-spored form of *Ophiobolus* producing perithecia on oats in the field.

The difference in spore length between *O. graminis* and *O. graminis* var. *avenae* was not affected by a change of host, and further support is given for the separation of *O. graminis* var. *avenae* from *O. graminis*.

Attempts to induce perithecial formation in culture by the use of various media, the addition of growth substances to them and ultraviolet light irradiation were unsuccessful.

Take-all is found on most cereal crops grown in mid-Wales, but attacks are usually slight and rarely cause concern to the farmer. As a result of a survey carried out in the summer of 1948, Davies (1950 *a*) came to the conclusion that the causal organism on oats, wheat and barley in mid-Wales is *Ophiobolus graminis* (Sacc.) Sacc. var. *avenae* E. M. Turner. On only one occasion did he find in this area what is considered to be the typical short-spored *Ophiobolus graminis* (Sacc.) Sacc., which was on a wheat crop in Montgomeryshire. In South Wales Hughes (1943), Davies (1950 *b*) and Jones (1951) reported *O. graminis* and *O. graminis* var. *avenae* occurring together on the same cereal crop.

Turner's (1940) separation of *O. graminis* var. *avenae* from *O. graminis* was based on differences in both spore length and host range. She found that whereas *O. graminis* attacked wheat and barley and its ascospore lengths ranged from 79 to 86μ, *O. graminis* var. *avenae* attacked oats in addition to wheat and barley and its ascospore length was 101–117μ. In her work several isolates of *O. graminis* var. *avenae* were used. These were collected from North and mid-Wales and slight differences in the pathogenicity of the isolates were observed. In cultural appearance, all isolates of *O. graminis* and *O. graminis* var. *avenae* were identical.

A study of the occurrence of *O. graminis* var. *avenae* in Scotland by Garrett & Dennis (1943) gave further support for the separation of *O. graminis* var. *avenae* from *O. graminis*, on the basis of mean ascospore length and host range. Their range of mean ascospore length, 96–119μ, was slightly greater than Turner's range. Garrett & Dennis drew attention to the fact that *O. graminis* var. *avenae* was always found in those areas in which oats were the main cereal crop.

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The purpose of this work was to investigate further the differences in the isolates of *O. graminis* var. *avenae* observed by Turner (1940) and to re-examine her separation of *O. graminis* var. *avenae* from *O. graminis*.

THE ISOLATION OF *OPHIOBOLUS* FROM OAT STUBBLE

Isolations of *Ophiobolus* were made from oat stubble collected at random in mid-Wales. Difficulties were encountered whilst isolating the fungus since often the ascospores failed to germinate and, even after germination, growth was slow and contaminations quickly overran the germ tubes and mycelium. It was found, for instance, that spores produced on winter oats in July 1952 germinated readily, whereas those from spring oats collected in September 1952 were stimulated to germinate feebly only after cooling the spore suspension overnight and incubating at 24° C. for 24–48 hr.

It was not always possible to make single ascospore isolations, because of the failure of the spores to germinate. On those occasions, single ascus isolations were made, since the ascospores germinated far more readily when remaining in the ascus. Single hyphal isolations were made from these ascus cultures as a substitute for single ascospore isolations. The usual hyphal tip method of isolation was unsuccessful, since such a small portion of a hypha failed to continue growing after it was severed from the mycelium, and a more laborious method was used. The periphery of an actively growing colony was examined under the binocular dissecting microscope. A hypha was selected and all the mycelium growing in its neighbourhood was removed with a needle. Careful observation was necessary to ensure that the selected hypha grew away from the colony, on its own. After several days, the hypha was severed as far away from the tip as possible and transferred to another plate of agar. If the hypha was severed too soon or too near the tip, it failed to produce a new colony.

The most satisfactory medium for spore germination was potato-dextrose agar, to which was added a few drops of 1 % lactic acid to suppress bacterial contamination.

DETAILS OF FUNGAL ISOLATES

Each of the seven isolates studied was given a serial number, by which it is referred to throughout the text. The information relating to each isolate is tabulated (Table 1).

The mean ascospore length of 88μ for OGA IV is within the accepted range of 70–90μ for *O. graminis*. This, to the best of the author's knowledge, is the first record of short-spored perithecia being found on oats in the field. On only one other occasion has the causal organism of take-all in mid-Wales been found to be the short-spored form of *Ophiobolus* and that, as previously mentioned, was on a wheat crop.

The isolate of *O. graminis* was supplied by Miss V. M. Wehrle of the National Agricultural Advisory Service at Bristol.

APPEARANCE OF ISOLATES IN CULTURE

The appearances of the isolates differed very noticeably. The types of growth can be classified into four distinct groups:

(1) The cultures of isolate IV and *O. graminis* were characterized by the formation of numerous thick, black strands, which radiated from the inoculum disk. The absence of aerial hyphae resulted in these strands appearing very distinct.

Table 1. *Details of* Ophiobolus *isolates*

Isolate no.	Date of isolation	Host	Area of origin	Mean spore length	Modal length	Range in length	Results of *pathogenicity tests on	
							Wheat	Oats
OGA I	Autumn 1951	Oat stubble	Breck-nock-shire	Typical long spores, but records lost	Unknown	Unknown	Weakly parasitic	Weakly parasitic
OGA II	Summer 1952	Oat stubble	Lampeter area, Cards.	As above	Unknown	Unknown	Weakly parasitic	Weakly parasitic
OGA III	Sept. 1952	Oat stubble	As above	103 μ	105 μ	95–110 μ	Actively parasitic	Actively parasitic
OGA IV	Oct. 1952	Oat stubble	As above	88 μ	88 μ	75–100 μ	Actively parasitic	Weakly parasitic
OGA V	Sept. 1953	Oat stubble	Traws-coed, Cards.	113 μ	120 μ	98–125 μ	Weakly parasitic	Actively parasitic
OGA VI	Sept. 1953	Oat stubble	Cardigan area, Cards.	113 μ	111 μ	100–125 μ	Weakly parasitic	Actively parasitic
O. graminis Bristol culture	Summer 1950	Wheat, growing crop	Bristol area	Short spores	Unknown	Unknown	Actively parasitic	Weakly parasitic

* Pathogenicity tests were carried out as soon as the isolations were made, since Russell (1939) found that isolates of *Ophiobolus* lost their pathogenicity when kept in culture. The spore lengths are those obtained from spores taken from perithecia found on stubble.

(2) Isolates III, V and VI produced dark mycelium and dark brown strands, but these were not so distinct as those of isolate IV and the *O. graminis* culture, and did not extend across the Petri dish. Some sparse white aerial mycelium was present in most of the cultures.

(3) Isolate II differed markedly in appearance from all other cultures; abundant white aerial mycelium and an occasional short, light brown strand were produced.

(4) The cultures of isolate I were jet-black in colour and only in very young cultures was it possible to distinguish strand formation. No aerial hyphae developed.

All the isolates grew well on a number of different media. The agars used were carrot, potato-dextrose, cornmeal, wheat, malt-extract and yeast-extract. Of these, carrot and malt were the most suitable for mycelial growth, but on all of them, with the exception of yeast-extract agar, the appearances of the isolates agreed with the descriptions given when grown on carrot agar. The use of yeast-extract agar for culturing resulted in the formation of more abundant aerial mycelium, which was of a lighter colour. Observations over a period of 3 years showed no marked changes in cultural appearance of the isolates.

STUDIES IN PATHOGENICITY

Seedlings were grown in sand culture and inoculated with agar disks (Garrett, 1936). The cultures were watered periodically with a wheat nutrient solution,* which permitted growth of the seedlings for 8–10 weeks. The following varieties of cereal seedlings were tested: S. 75, S. 84, S. 171, S. 225, Grey Winter and Victory oats, Atle wheat and Spratt Archer barley.

The fungal isolates differed in virulence, but the order of degree of infection in which the different cereal varieties could be arranged were approximately the same for different fungal isolates, with the exceptions noted below. Isolates I, II and IV were weakly parasitic and isolates III, V and VI were actively parasitic, on the oat varieties. On wheat, isolates III and IV were the only ones which were actively parasitic.

Isolate IV, although obtained from oats, behaved in the same way as did the culture of *O. graminis*. It did, however, develop a few perithecia on oat seedlings, and the ascospores had an average length of 80μ. Turner (1940) found that oats were weakly parasitized by *O. graminis*, and that a few perithecia developed on the leaf sheaths of oat seedlings under experimental conditions.

FORMATION OF PERITHECIA

Isolates I and II did not form perithecia on wheat or oat seedlings. Isolates V and VI produced perithecia on oats but not on wheat, whereas isolate III produced numerous perithecia on oats and wheat. Both isolate IV and the *O. graminis* culture from Bristol produced perithecia on wheat seedlings but IV also formed a few on oats and this is the only way in which it differed from the Bristol culture. The spore lengths are shown in Tables 2 and 3. It can be seen that, throughout, these remained reasonably constant, even on the different oat varieties.

Table 2. *Ascospore lengths of* Ophiobolus *isolates infecting wheat and oat seedlings grown in sand culture*

	O. graminis culture	Isolate III		Isolate IV		Isolate V	Isolate VI
Host ...	Wheat	Wheat	Oats	Wheat	Oats	Oats	Oats
Mean length (μ)	75	114	119	77	80	111	122
Modal length (μ)	75	118	120	80	80	110	125
Range (μ)	62–87	103–130	100–135	65–87	60–97	100–125	105–138

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* Wheat nutrient solution contained 1 ml./l. M ammonium acid phosphate; 6 ml./l. M potassium nitrate; 4 ml./l. M calcium nitrate; 2 ml./l. M magnesium sulphate and 1 ml./l. 0·5 % iron tartrate.

Most of the perithecia produced on the seedlings grown in sand cultures were found on the roots of the plants and not, as under natural conditions, on the leaf sheaths. They occurred in greater numbers on those roots pressed against the surface of the glass pots in which the seedlings were grown than on those growing in the centre.

Table 3. *Ascospore lengths of* Ophiobolus *isolates grown on different oat varieties*

		Oat varieties			
		Grey Winter	S. 84	Victory	S. 75
Isolate VI	Mean length (μ)	120	122	120	121
	Modal length (μ)	125	125	122	123
	Range (μ)	105–138	105–138	105–135	105–135
Isolate V	Mean length (μ)	102	111	111	110
	Modal length (μ)	100	110	113	113
	Range (μ)	85–125	100–125	95–125	95–130
Isolate III	Mean length (μ)	—	128	122	—
	Modal length (μ)	—	125	123	—
	Range (μ)	—	100–150	100–150	—

ATTEMPTS TO INDUCE PERITHECIAL FORMATION IN CULTURE

Considerable stress is placed upon ascospore length in Turner's (1940) separation of *O. graminis* var. *avenae* from *O. graminis*. Davies (1950*a*) found a wide range of ascospore length even within a single ascus, and a larger one still (80–130μ) within a single perithecium of *O. graminis* var. *avenae*. Attempts were therefore made to induce perithecial formation in culture, so as to study the plasticity in ascospore length and also any other differences between isolates.

Nutrition. This has been found by many workers (Hawker, 1936, 1939*a*, *b*, 1942; Barnett & Lilly, 1947*a*, *b*) to influence the sporulation of fungi. Many different culture media were tried but perithecia were formed only on carrot agar inoculated with isolate I. These developed 18 months after the isolation was first made. Cultures of this isolate retained the ability to produce perithecia on carrot agar for only 3 weeks, even on freshly prepared agar, and then perithecial formation ceased as suddenly as it had started. The perithecia were of the *O. graminis* var. *avenae* type.

Cereal seedlings were grown on vegetable agars inoculated with *Ophiobolus*; the concentration of the culture media was varied; biotin, thiamin and oat extract were added to the media and the isolates were grown in mixed culture, but perithecia did not develop.

Light. Irradiation of some fungi with ultra-violet light occasionally has a stimulating effect upon sporulation. Charlton (1953) successfully used ultra-violet light to stimulate sporulation in *Alternaria solani*. His methods were used with *Ophiobolus* and comparison was made between staggered and continuous irradiation for periods of 2–40 min. and at different intensities. Irradiation, even for short periods, was found to check mycelial growth in all cultures and, after treatment-periods of more than 8 min., knots of hyphae were induced in the position occupied by the margin of the growing colonies at the time of irradiation. These knots looked very like perithecia but the hyphae, although distorted, were found to be undifferentiated. These knots of hyphae were white at first and then became black. They might have been perithecial initials. Isolates were also irradiated on media containing biotin and thiamin, but the hyphal knots which developed remained undifferentiated.

Temperature. Petri dishes of carrot agar were inoculated with the isolates and incubated at temperatures of 4, 14, 21·5, 24, 26·5, 30 and 37°. The rate of growth varied considerably at the different temperatures, the optimum being between 21·5 and 24° for all isolates. An interesting result was the formation of mature perithecia on the cultures of isolate I, incubated at 14°. They appeared when the mycelium had filled the Petri dishes, 24 days after inoculation. The perithecia formed at the periphery of the Petri dishes and were very similar macroscopically to the hyphal knots that developed after irradiation. The ascospores were of the *O. graminis* var. *avenae* type with respect to length (mean 104μ, modal 101μ, range 87–117μ). The other isolates failed to produce perithecia, even when low temperatures were coupled with irradiation and a range of different media.

I should like to express my thanks to Dr Lilian E. Hawker, Dr T. Whitehead and Dr D. L. G. Davies for their advice and guidance in this work and to Mr W. Morgan for his practical assistance with the experimental work. Valuable information on the investigational work being carried out on the take-all disease of cereals in South Wales was supplied by Dr J. B. Jones. The material presented here formed part of a thesis for the M.Sc. degree in the University of Bristol.

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INVESTIGATIONS ON A DISEASE OF *COFFEA ARABICA* CAUSED BY A FORM OF *COLLETOTRICHUM COFFEANUM* NOACK.

III. THE RELATION BETWEEN INFECTION OF BEARING WOOD AND DISEASE INCIDENCE

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(With 9 Text-figures)

By the use of certain techniques, the inoculum production by different portions of the bearing wood of coffee trees can be determined. Conidia from infected fruit were found to play only a minor part in the spread of coffee berry disease; conidial production from bearing wood is responsible for the level of disease in any season. The region of maximum crop density on the bearing wood coincides with that of maximum inoculum production. Control measures directed not at protecting the developing fruit, but at reducing the amount of inoculum produced by the branches, give satisfactory results. The importance of this in relation to control measures, and to the geographical spread of the disease, is discussed.

In a previous communication (Nutman & Roberts, 1960*a*) it was shown that the causal agent of coffee berry disease, *Colletotrichum coffeanum* Noack. (*Glomerella cingulata* (Stonem.) Spauld. & Schrenk), habitually colonizes the semi-moribund tissues external to the developing phellogens in the twigs of *Coffea arabica*. A series of these phellogens, generally three to four, is formed and the tissues external to the most deeply seated one are normally invaded by the fungus, which forms its acervuli on the surface of the twig. This was at first thought to be of importance only in connexion with the maintenance of a reservoir of infection in the trees during periods when the berry crop was absent. The distribution of infected berries on the twig, however, together with the difficulty in accounting for other field observations in terms of an epiphytotic build-up on the fruit, suggested that bearing wood might be of greater significance as a source of inoculum than had been thought. In consequence, the following investigation was undertaken.

TECHNIQUE

The technique used throughout these studies consisted in removing, by repeated washing, all superficial contamination and conidia from the part of the branch under investigation. The sample was then incubated, usually for 24 hr., under standard conditions and the conidia produced during the period were removed by shaking with a known volume of water. An aliquot of the resulting suspension was then centrifuged, and the pellet resuspended in a known volume of water. The conidial concentration was then determined by haemocytometer counts.

During the early phases of this work, conidial production was related, for example, to given lengths of entire branches. Later, for reasons which will become apparent, the technique was standardized as follows. Samples consisted, as a rule, of twenty branches, one being taken at random from each of twenty trees. The distal ends were discarded, being severed at the node below which the first sign of maturation, i.e. yellowing, appeared in the green epidermis; the immature tissues above this node do not become colonized by the fungus. This discarded portion was usually three to six internodes in length; a second cut was made eight internodes down, and the remainder discarded. The 7th or 8th internode was usually the first to show true bark, with the sloughing off of the dead cortical tissues (Fig. 3). Phelloderm is not colonized by the fungus, and consequently no conidia are produced on the bark.

The samples were then divided into their separate internodes, and were placed in eight serially numbered screw-top jars, the first jar, for example, containing all twenty no. 1 internodes. After washing and draining, these were incubated at 22° C., the optimum temperature, for 24 hr. (Nutman & Roberts, 1960*b*). Twenty ml. water were then added to each jar and, after shaking, 10 ml. of the resulting suspensions were centrifuged to firm pellets. One drop of cotton blue in lactophenol was added to each, and the volume brought up to 1 ml. Haemocytometer counts of conidia were then made, always in duplicate by two observers. The total length of the component parts of each sample was measured and their volume determined by displacement. This enabled the total surface area of each sample to be calculated, and the average production of conidia over the 24 hr. period was expressed as conidia/cm.^{2}/hr. This figure will be referred to hereafter as 'inoculum-potential'; 'maturing wood' is defined as that portion of the twig between the point at which the green epidermis is acquiring the yellow colour of ripening, and the point at which true bark commences.

From time to time, some of the conidial suspensions were inoculated to green coffee berries to test pathogenicity. Samples were also collected from disease-free plantations, and treated in a similar manner: in no case was the pathogen recovered. It was observed, however, that the rate of conidial production by the non-pathogenic form was often appreciably greater than that by the pathogen.

INOCULUM PRODUCTION BY FRUIT AND BY BRANCHES

Of the many assessments made, the following are typical. In a plantation which had, at the time, relatively few diseased berries, the average tree (estimated from a study of fifty) was found to have seventy-two diseased immature berries, and twenty-four diseased ripe ones among the healthy crop. The length of the maturing wood was estimated at 12 m. Later in the same month, when the number of diseased berries was much higher, one branch was collected from each of twenty trees and the conidial production from all the diseased berries and from the maturing fruit was assessed. The results of both samplings are given in Table 1, and show that the contribution by the fruit to the total amount of inoculum is comparatively low, being, in the second and higher sample, less than one-fifth. Similar determinations made at different stages of crop maturity, and at different disease-intensities, have given similar results. Under exceptional conditions, however, the relative amount of inoculum produced by the crop may be higher than in sample 2, but it has never been found to assume a major proportion. The larger amount of conidial production from the maturing wood is, of course, because its area is very much greater than that of the diseased fruit. A severely attacked ripe berry produces about 900 conidia/cm.^{2}/hr., and a green berry about 680. As will be seen later, conidial production from wood is variable, but is at least 150/cm.^{2}/hr. On one occasion it was estimated that an average bearing tree had 270 cm.^{2} diseased fruit, and 9730 cm.^{2} maturing wood. The calculated inoculum production would be 10·5 % from the fruit, and 89·5 % from the wood: these figures are comparable with those shown in Table 1.

Table 1. *Conidial production in millions of an average tree, expressed as conidia extruded per hour, at two different levels of disease incidence*

	Sample no. 1		Sample no. 2	
	Total no.	% total	Total no.	% total
Young twigs	64·5	70·3	88·0	75·8
Old twigs	24·5	26·6	6·3	5·6
Fruit	2·8	3·0	21·6	18·6

Table 2. *Rate of conidial production* (*conidia/cm.^{2}/hr.*) *by branches in relation to their degree of maturation*

Sample no.	Green (hr.)			Semi-mature (hr.)			Mature (hr.)		
	0–24	24–48	48–72	0–24	24–48	48–72	0–24	24–48	48–72
1	1·6	1·9	3·9	19·6	95·0	120·0	5·2	17·9	16·2
2	1·5	3·3	4·8	32·4	194·6	227·0	6·6	23·2	23·3
3	1·0	1·8	1·9	16·8	72·0	96·0	5·9	13·1	19·7
Mean	1·3	2·4	3·2	22·6	120·9	144·3	5·8	18·1	19·7

CONIDIAL PRODUCTION IN RELATION TO DEGREE OF MATURATION OF WOOD

Three ten-branch samples, each consisting of one branch from each of ten trees, were collected and divided into portions consisting of the green immature wood, the maturing wood, and the wood showing true bark. The nine batches were incubated separately and conidia were harvested from each after three successive periods of incubation. The results are summarized in Table 2, where it can be seen that conidial production is, in the main, confined to the ripening wood and, as would be expected, the rate increases with time.

Studies of inoculum production by different parts of the twig were made in more detail by sampling each internode separately. The first internode was, as usual, that at which the first signs of maturation were evident, and the last was the 8th proximal to this. This was usually the first, or sometimes the second, having true bark. It sometimes happened that, after a rapid growth-flush, the 8th internode was still only semi-mature, but this was unusual. Internode samples from twenty branches were bulked for processing. Fig. 1 illustrates the combined results from thirty-five such assessments, each point on the curve representing the average inoculum potential of 700 internodes (thirty-five samples of twenty). The same figure also shows the results of two individual assessments—the first at a period of the year when inoculum production was high, and the second when it tended to be low. It is apparent that the maximum is located at the 3rd and 4th internode, and that conidial production falls to a very low level at the 1st and at the 8th.

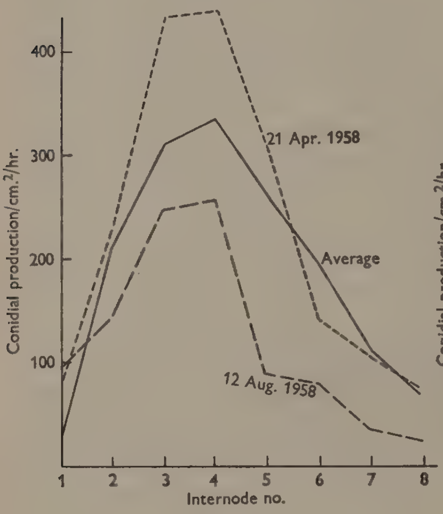

Fig. 1

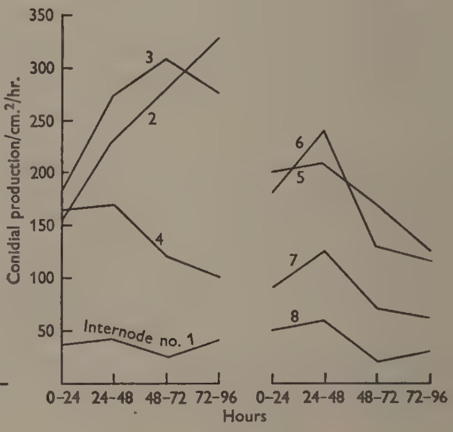

Fig. 2

Fig. 1. Conidial production from maturing wood.

Fig. 2. Conidial production from each internode over four successive 24 hr. periods.

Fig. 2 illustrates conidial production over four successive 24 hr. periods for each internode. The 1st internode, where colonization by the fungus has just begun, and only a few acervuli are present, shows little change with time. The 2nd, where there are many both mature and developing acervuli, shows a steady and marked increase. The 3rd, with an initial rate higher than the 2nd, rises for the first 3 days and then falls, probably as the result of exhaustion of food reserves. The other internodes (all of which have been, in the past, capable of high conidial production) exhibit on the whole a more or less steady decline with time, clearly the consequence of an exhausted mycelium and lack of food reserves.

POSITIONAL RELATIONSHIP BETWEEN CROP, INOCULUM POTENTIAL, AND DISEASE INCIDENCE

The berries of *Coffea arabica* are borne on branched peduncles at the nodes and, as can be seen in Fig. 3, crowded clusters of them may almost surround the twig in close proximity to the acervuli on it. It seemed likely, therefore, that the relation between distribution of berries along the twig and the inoculum potential of adjacent internodes would be of significance. Fig. 4 gives one example in illustration of this relationship. It shows that the position of maximum inoculum potential coincides very closely with the region of maximum crop concentration. Fig. 5 illustrates conidial production in relation to position of crop in a slightly different manner; it shows average figures for both crop-concentration and inoculum potential obtained from eight monthly assessments at one particular site. To compare the amount of crop at any given node with the average inoculum potential over the period the following procedure was adopted: at the time of the first assessment, internode no. 1 on twelve branches on each of twenty trees was labelled. Each successive monthly sample comprised one branch from each tree, and each, therefore, included the eight internodes older than the labelled ones. During the 8 months, maturation of the younger internodes proceeded, and the labelled internode was, towards the end of the period, in position 2 or 3. For this reason, the average

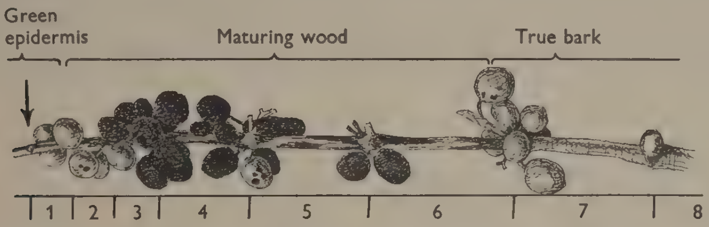

Fig. 3. Fruiting branch of *C. arabica* illustrating distribution of healthy and diseased berries. *N.B.* Some berries adjacent to internodes 5 and 6 have fallen.

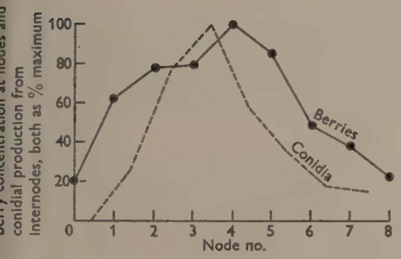

Fig. 4

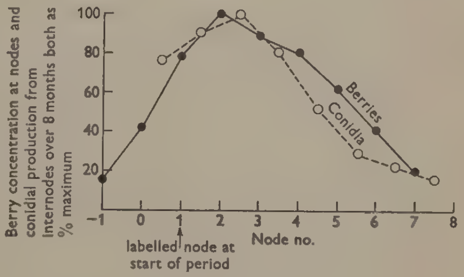

Fig. 5

Fig. 4. Relation between spore concentration and crop concentration.

Fig. 5. Relation between crop concentration and conidial production over 8 months. For explanation see text.

inoculum potential is higher at the distal end and lower at the basal end than in Fig. 4. In Fig. 5 it can be seen that average maximum inoculum potential coincides very closely with the position of maximum crop concentration. It is, therefore, to be expected that a higher proportion of disease will occur at this point. In the field it can be seen that disease incidence is usually greatest at the point of highest inoculum potential while, as is illustrated in Fig. 3, the crop adjacent to the 1st, 2nd, 7th and 8th internodes is comparatively unaffected.

Epiphytological studies by Bock (private communication) confirm that maximum infection occurs at the point of maximum inoculum potential, and also that this region moves outwards along the twig as the wood matures, i.e. as the point of maximum inoculum potential progresses apically.

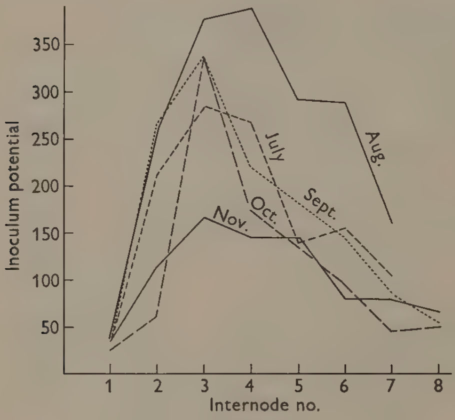

Fig. 6

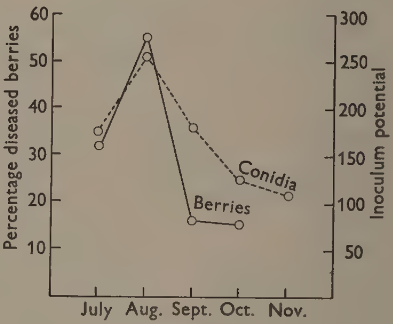

Fig. 7

Fig. 6. Conidial production, West Rift, August–November 1958.

Fig. 7. West Rift. Relation between crop disease and average inoculum potential.

Fig. 6 illustrates inoculum-potential curves over 5 successive months at one site: when these are integrated for each month and the total inoculum potential compared with the recorded average disease incidence at the same time and from the same branches, as in Fig. 7, it is clear that the proportion of disease in the crop bears a close relationship to the infection potential of the branches.

Disease control in relation to inoculum potential

From the foregoing it seems probable, first, that infection of the crop derives, in the main, from conidia produced on the bearing wood; secondly, that where climatic conditions are not limiting, the degree of infection is related to the amount of inoculum produced by these branches. It should, therefore, be possible to effect control of the disease by applying treatments which might either protect the developing bearing wood, or reduce inoculum production from it. The conidial concentration necessary to infect a berry is very high (Bock, 1960; Nutman & Roberts, 1960*a*); consequently a modest reduction in inoculum might be expected to have a considerable effect, since if an infection-drop has a conidial concentration less than 10^5 conidia/ml. the chances of it producing a lesion would be small.

Since 1956, a continuous series of field experiments has been conducted in various parts of Kenya; these have included trials of a wide range of fungicides in addition to experiments on the effects of treatment at different seasons. Thus a range of material was available for study. With detailed records available of the level of control achieved by any given treatment, it was possible to relate the reduction in inoculum potential, as measured in the laboratory, to the observed control.

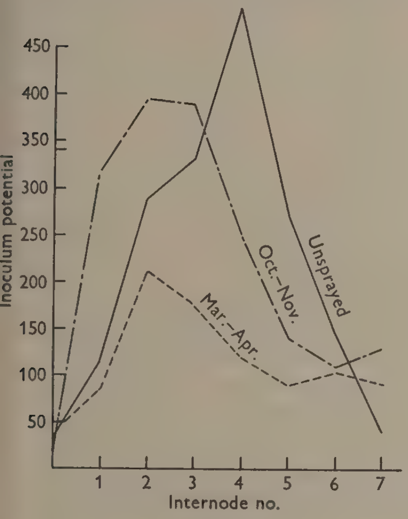

Fig. 8

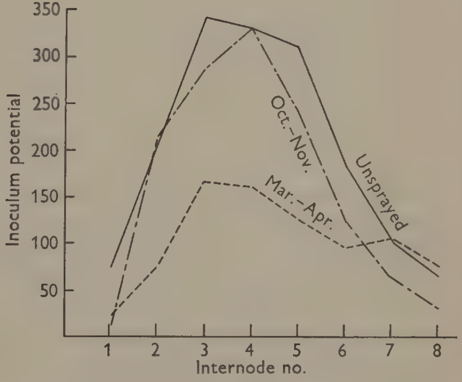

Fig. 9

Fig. 8. West Rift. Conidial production from unsprayed branches compared with that from those receiving effective (March–April) sprays and ineffective (Oct.–Nov.) sprays.

Fig. 9. East Rift. Conidial production from unsprayed branches compared with that from those receiving effective (March–April) sprays and ineffective (Oct.–Nov.) sprays.

During 1958, twenty-two assessments of various trials sited both east and west of the Rift were made. Of these, fourteen were from blocks where yield records showed the achievement of effective control. The average reduction in inoculum potential of these blocks was 61 %, and, in one case, 91 %. Of the eight where the treatment proved ineffective in controlling the disease, the average reduction was by less than 2 %. Figs. 8 and 9 illustrate inoculum potential on two plantations, one east and one west of the Rift; both show that a treatment, ineffective because applied at the wrong time, has not significantly reduced inoculum potential, whereas a correctly timed one has done so. It is noteworthy that in both these examples the effective treatments are those applied just before and during the period of growth-flush.

A further and more detailed study was made by sampling every plot in a 2^5 replicated trial comprising sixty-four plots, each of twenty trees. One branch was taken at random from each tree, and the incidence of disease and the inoculum potential determined. The size of the samples rendered the assessment more difficult than usual, because of the difficulty in washing with the requisite thoroughness. Consequently, as can be seen in Table 3 below, the error in the haemocytometer counts is higher than is desirable. It is clear that the treatment which has given the highest significant effect on the inoculum potential is also the one which has resulted in maximum control.

Table 3. *Main effects of fungicidal treatments on inoculum potential of maturing wood and on disease incidence*

Lay-out: 2^5: twice replicated. Sixty-four plots each of twenty trees.

Treatments	*a*	*b*	*c*	*d*	*e*	
Time of application ...	Nov.-Dec.	Jan.	Feb.	Mar.	Apr.	
No. sprays ...	4	2	2	2	2	
Effects	A	B	C	D	E	S.E.
Inoculum potential	100	44	154**	18	5	55·6
Disease incidence	2·9	24·2**	30·0***	2·3	1·8	7·9

Discussion

It has been reported previously (Nutman & Roberts, 1960*a*) that the pathogenic form of *Colletotrichum coffeanum* probably arose as a mutant a short time prior to 1922. Before that, a group of non-pathogenic forms was present (Small, 1926) and there are some indications that a very mild pathogen, capable only of infecting ripe berries, also occurred in some districts (Macdonald, 1926). It also seems that the pathogenic form, when it arose in one particular district, displaced the non-pathogen, and in regions where coffee berry disease has been present for a long time the cortical tissues of the bearing wood now contain only the former.

From the district of origin, the pathogen spread to most of the coffee-growing districts in Kenya above certain altitudes. On field evidence it seems eventually to reach a boundary zone where infection is confined to highly susceptible varieties, to flowers, or to small areas where topography or cultural practices make the local climate approximate to that of the higher regions. It was also suggested that limits to the ultimate spread of the disease were set by the critical requirements, with respect to humidity and temperature, for invasion by the fungus. It was concluded that in plantations at the lower altitudes (below about 5600 ft.) the pathogen is unable to invade the fruit because here these requirements are not met (Nutman & Roberts, 1960*b*).

The non-pathogenic form, however, is present at these, and yet lower, altitudes and it has now been shown that its rate of conidial production on the bearing wood is sometimes, at least, greater than that of the pathogen. The question thus arises as to why the distribution of the saprophyte should be wider than that of the presumably more efficient parasite; this question is still unsolved. A possible explanation is that the former might be able to penetrate the semi-moribund cortical tissues in a shorter time than can the parasite. Macdonald (1926) showed that there was a marked difference between the parasitic strain and the saprophytic ones with regard to appressorial formation, the latter forming these very sparingly. The surface of the maturing cortex, because it has ceased to grow while the twig itself is still increasing in diameter, becomes fissured and penetration by germ-tubes could obviously take place through these cracks. It seems at least possible that direct penetration in this way might be more rapid than by appressorial formation followed by germination of the appressorium; if this were so the distribution of the saprophyte could well be broader, if it were able to penetrate fissured epidermis where suitable temperature and humidity conditions do not persist for long enough for invasion by the parasite.

When invasion of the cortex has once occurred the tree is then permanently colonized. The growth of the fungus can keep pace with the apical progress of maturation. No humidity limitations affect this development, and at all altitudes part of every day would provide suitable temperature conditions for rapid mycelial growth.

As regards conidial production on the bearing wood by the pathogen, the evidence presented supports the view that this is the main source of the inoculum for both flowers and fruit and that only in the later stages of an outbreak do the conidia produced on diseased fruit play any significant part. Not only is the level of disease incidence in the fruit at nodes of different ages correlated with the inoculum potential of adjacent internodes (Figs. 4 and 5), but the amount of disease in the crop at different times during its development is related to the average inoculum potential of the bearing wood at the same times, as is illustrated in Fig. 7. Moreover, it is only those chemical treatments which demonstrably reduce inoculum potential which are effective in reducing disease in the crop. Additional field evidence for the importance of the role of the maturing wood is afforded by the consequences of certain cultural treatments, namely, stumping the trees to just above ground level, or pruning so severely as to remove all but the very mature old wood, thus leaving no local source of inoculum. It is common experience that trees so treated often remain free from disease for some time.

As regards flowers, these are highly susceptible to attack (Nutman & Roberts, 1960*a*) and considerable losses are sometimes experienced. Flowers are often, though not invariably, produced when there is little or no crop on the trees and in such cases infection can only derive from inoculum produced on the wood. Despite the extreme susceptibility of flowers, serious losses are rarer than might be expected. It was suggested that the reason for this might be their ephemeral nature, for only rarely would the temperature and moisture requirements for invasion coincide with flowering. While this may be, in part, the explanation, the distribution of flowers on the twig may also contribute. They are produced, in general, on those parts of the twig distal to the maturing wood, that is, above internodes 2 or 3, and it has been shown that inoculum potential here is low or even at zero. The source of inoculum for an attack on the flowers is probably the bearing wood of other branches, from which conidia might be carried by rain-splash or drip.

Reference has been made (Nutman & Roberts, 1960*b*) to direct invasion of berries by mycelial growth from the branch. This is rare, but when it does occur spectacular losses of crop can result. The developing phellogens normally limit the internal proliferation of mycelium, and these interpose an effective barrier to invasion of either the central stele or the peduncle. Under certain conditions penetration of the phellogen can result in the condition known as Elgon dieback and similarly penetration of the phellogen between the cortex and the peduncle can result in the fruit becoming invaded from the base. This phenomenon is more common in districts with high and well-distributed rainfall where rapid growth of the branches is common; in such conditions the phellogen barrier may become less resistant to penetration.

The occurrence of the pathogen in maturing wood affords an explanation for the way in which the disease has crossed geographical barriers which would seem, at first sight, to be insuperable as the fungus is distributed by rain-splash, and its conidia are highly sensitive to desiccation. It should be recalled that the disease was first recognized west of the Rift in 1922 (Macdonald, 1926) and was confined there until 1939. In that year it was recognized in the Mt. Kenya region, a jump of at least 100 miles across the Rift Valley. Twelve years later it appeared in Upper Kiambu, another jump of 100 miles.

Coffee plantations are normally established and supplied by nursery-grown seedlings, generally 18–24 months of age, and old enough to contain maturing wood. From such seedlings, raised in areas where the disease is prevalent, the pathogen has frequently been isolated. Commercial production of seedlings is common, and plants are often transported over very considerable distances. There seems little doubt that movement of the pathogen from one district to another has been effected in this manner. Additional support for this conclusion is afforded by the extremely sporadic occurrences of the disease during the past year in African holdings on Mt. Kenya. All the field evidence, in fact, is against any supposition that it can spread any considerable distance without the intervention of man.

Recognition of the major role of the bearing wood as a source of inoculum permits of a rational approach to the problem of control. To maintain an effective fungicidal coating, over many months, on berries which are continually increasing in size, is difficult: it seems possible therefore, that control might be more readily effected by reduction of inoculum potential. Applications of fungicide at a specific time of the year will appreciably reduce inoculum potential for a considerable period, and these treatments, though applied at a time when there may be no crop on the trees, effectively reduce disease. As an example, Bock (private communication) has recorded an instance where crop on untreated trees was reduced by disease to the low figure of 2 cwt./acre, whereas trees treated at the critical period of growth-flush yielded 14 cwt., an increase of 700 %.

Reduction of inoculum potential should also have an effect on Elgon dieback, caused by invasion of the central stele of the twig (Nutman &

Roberts, 1960*a*). Although this does not normally reach economically serious proportions, it has in the past been readily observable on many plantations. In recent years many of these have adopted routine spraying at the time known to cause maximum reduction in inoculum potential, and on such plantations this disease is now rarely seen.

We acknowledge with pleasure the co-operation of the Managements of many coffee plantations, especially those of Savani, Lilloch, Mweiga, Kentmere, and Yara. Mr K. R. Bock, of this Unit, has kindly provided us with data on the effects of fungicidal sprays on crop production.

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STUDIES ON BRITISH CHYTRIDS

XIX. ON *PHLYCTIDIUM APOPHYSATUM* CANTER EMEND.

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(With Plate 34 and 3 Text-figures)

Phlyctidium apophysatum Canter parasitic on *Mougeotia* is redescribed with further details of its morphology and development and given an emended diagnosis. A sexually formed resting spore was found.

Since a brief account of this chytrid was first given (Canter, 1947) it has been found in the tarn at Tarn, near Silloth, Cumberland (coll. January 1951 and May 1954). Material from this locality was put into a pond in my garden at 'Ellerbeck', Ambleside, Westmorland, where subsequently the chytrid was collected in May 1960. In all the collections the hosts were species of *Mougeotia*. Studies based on the more recent and abundant material show that the original description must be revised. Specimens from Tarn and 'Ellerbeck' agree well and this account (except where noted) embraces both localities.

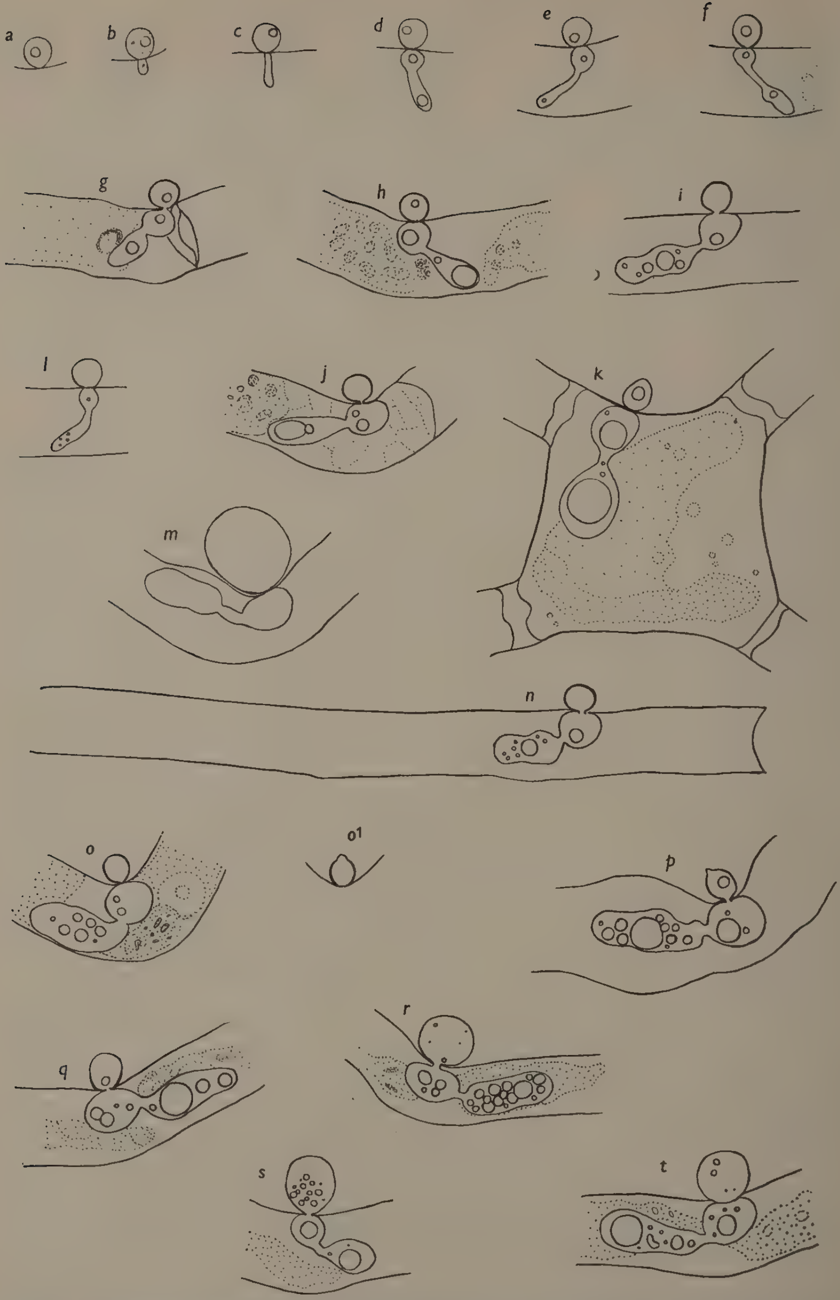
The zoospore settles and encysts on the wall of a *Mougeotia* cell. A germ tube from it penetrates the wall and extends for some distance inside (Text-fig. 1 *b*, *c*; Pl. 34, fig. 1). On further growth the proximal portion becomes more swollen and spherical than the distal part, thus giving rise to a constricted part between them. The proximal part is destined to develop into the prosporangium, while the absorbing organ develops from the more tubular distal part. The rhizoid is sac-like, unbranched and at maturity, in the majority of specimens, connected to the prosporangium by a narrower isthmus region (Text-fig. 1 *m*). In the very early stages of development the rhizoid portion is at right angles to the length of the *Mougeotia* cell, but during growth it curves and at maturity usually lies in a direction parallel to the long axis of the host-cell (Text-fig. 1 *n*–*p*). When young the zoospore cyst, prosporangium and rhizoid each contain one refractive globule (Text-fig. 1 *d*–*g*). As development proceeds the globule usually disappears from the zoospore cyst, whose wall thickens and becomes refractive (Pl. 34, fig. 3). Large globules accumulate in the prosporangium and apophysis. Development is endo-exogenous and only when the internal system is fully grown does the zoospore cyst begin to expand into the sporangium. The emergence of the sporangium wall is first seen as a thin-walled hyaline bulge at the apex of the zoospore cyst (Text-fig. 1 o^1, *p*). As the sporangium enlarges pieces of thick zoospore case wall remain at its base (Text-fig. 1 *q*). At first the bulge is spherical (Pl. 34, fig. 6) and the protoplasm contains few globules. As growth proceeds many sporangia become ovoid, being slightly broader at the base than at the apex (Pl. 34, fig. 7) and globules of unequal size accumulate in the protoplasm. Later the globules become smaller, all of approximately equal size, and they appear to be arranged in tiny clusters in a definite pattern (Text-fig. 2*e*). These small globules then merge together again to form the single large globules which indicate the positions of the zoospores (Pl. 34, fig. 9). Some of the sporangia which appear to be mature have very large globules (Text-fig. 2*f*–*i*); others have slightly smaller ones (Text-fig. 2*k*, *l*). However, if one wishes to see dehiscence it is not advisable to watch a sporangium which contains very large globules. It thus seems likely that the zoospore globules become slightly smaller after their formation and before dehiscence. After the sporangium is full-grown the rhizoid and prosporangium collapse and shrivel (Text-fig. 2*k*, *l*). Eventually they become almost unrecognizable.

On dehiscence the apex of the sporangium dissolves and some of the protoplasm and globules flow out as an undifferentiated mass surrounded by a thin-walled vesicle. There is no doubt that a membrane surrounds the extruded protoplasm. Text-fig. 2*t* is of a specimen in the stage shown in Text-fig. 2*o* after squashing. The extent of this mass is limited (Text-fig. 2*m*–*o*) and the process is similar to that described for *Rhizophydium sphaerocarpum* (Zopf) Fischer. Whether the membrane is formed from an inner wall of the sporangium is unknown. Gradually the outlines of the zoospores can be seen, although still forming a motionless mass. At last one breaks through the membrane and movement is initiated. A large part of the thin membrane then dissolves, but since the zoospores are entangled by their flagella dispersal is slow and sporadic.

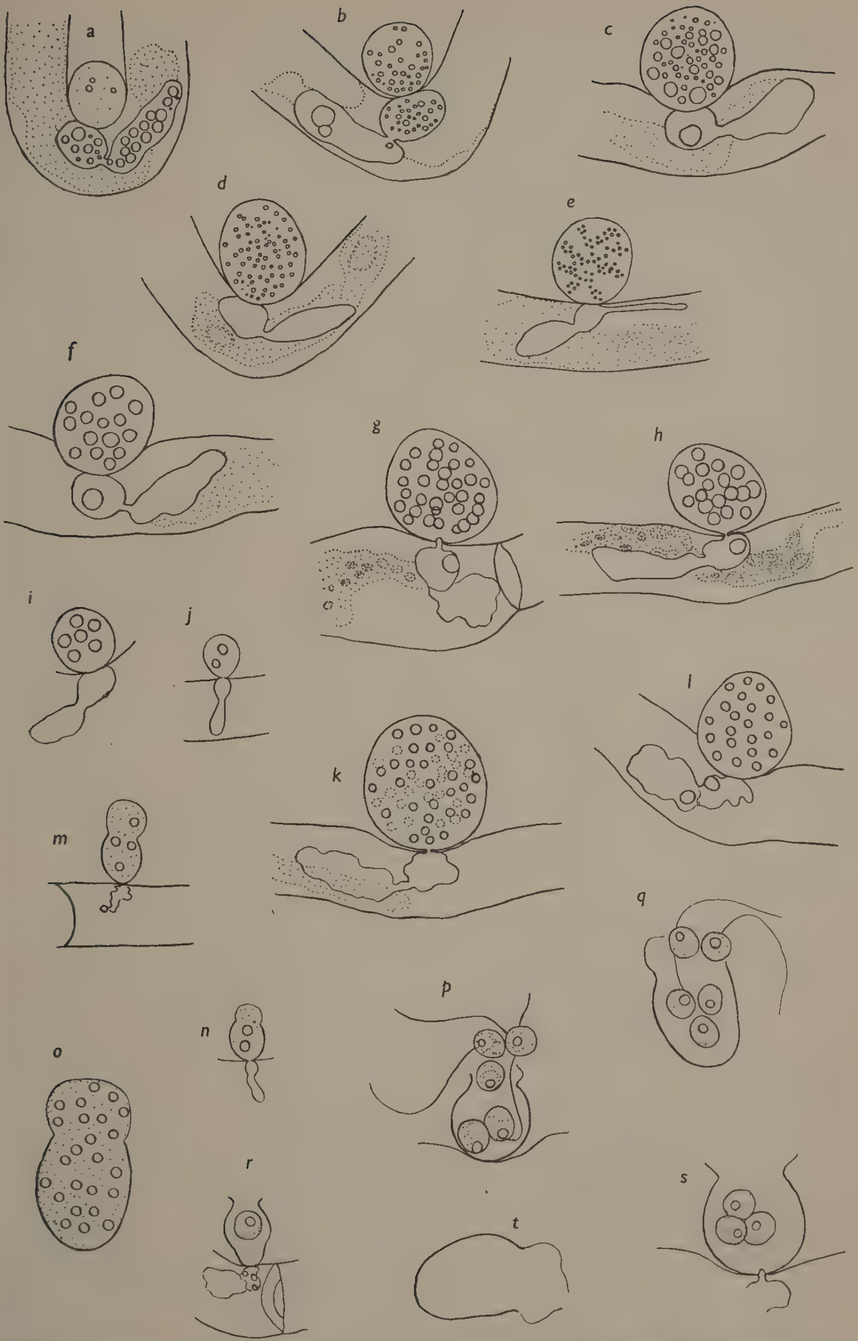
The zoospore has a large basal globule, above which is a vacuolate region. Movement is mainly smooth gliding with occasional darting or hopping, especially when changing direction or upon meeting an obstacle. The wall of the empty sporangium is persistent and stains well in aqueous cotton blue. Often the remains of membrane or vesicle can be seen as thinner-walled prolongations continuous with the apex of the empty sporangium (Text-fig. 2*p*–*s*). In large sporangia a convex ingrowth of wall material at the juncture between the sporangium and prosporangium can sometimes be seen (Text-fig. 2*s*).

The chytrid thalli vary greatly in size. Small ones develop on the thinner species of *Mougeotia* and when the zoospore germinates at a distance from the chloroplast region. Again, they occur on cells where the chloroplast has gone into a developing zygospore (Text-fig. 1*n*). There are no outstanding colour changes in the algal chloroplast which becomes reduced to an amorphous green mass. Curvature of the host cell at the point of insertion of the chytrid is common. Very rarely was a thallus found infecting an immature zygospore (Text-fig. 1*k*).

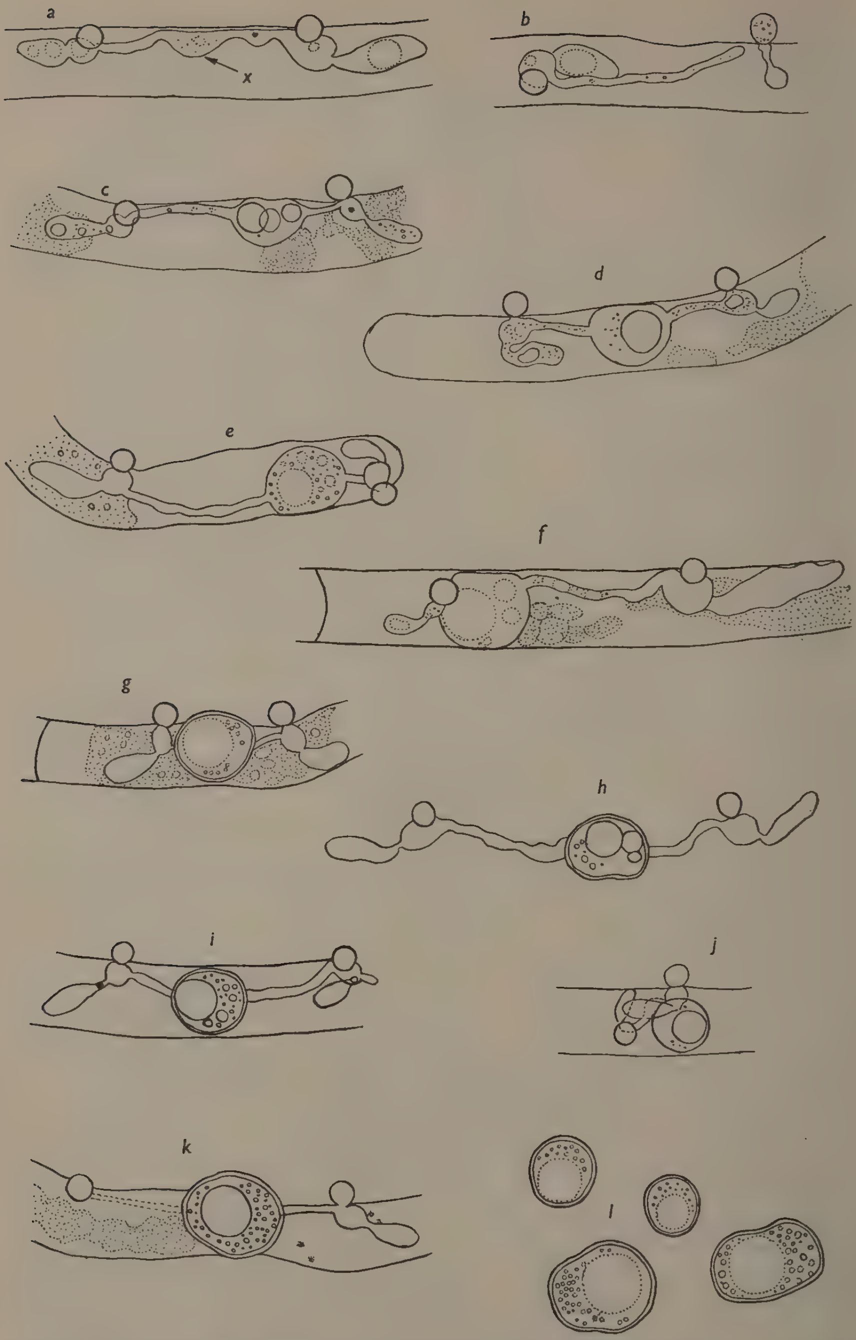
Material containing resting spores was found once in a collection from Tarn (May 1954) and unfortunately specimens were not abundant. In the earliest stage in its formation (Text-fig. 3*a*) two thalli each consisting of an epibiotic empty encysted zoospore case and an endobiotic swelling and tubular rhizoid are connected by a conjugation tube. As the swelling (*x*) which will develop into the resting spore increases in size large globules appear in it. Later it becomes surrounded by a thick refractive colourless



Text-fig. 1. *Phlyctidium apophysatum*: *a*, encysted zoospore; *b*, *c*, development of wide germ tube; *d–k*, formation of prosporangium and enlargement of rhizoid; *l*, thallus in region devoid of chloroplast, note poor development of endobiotic parts; *m*, specimen in glacial acetic acid, isthmus between prosporangium and rhizoid clearly visible; *n*, thallus in a cell whose content has passed into a zygospore; *o*, thallus with endobiotic system fully grown; o^1, same as *o*, some time later, the zoospore case has a papilla-like bud, the beginning of the sporangium; *p–t*, stages in growth of the epibiotic sporangium. × 1050.



Text-fig. 2. *Phlyctidium apophysatum*: *a–d*, stages in emptying of prosporangium and rhizoid with consequent growth of sporangium; *e*, sporangium with globules in the 'cluster' stage; *f–i*, sporangia with large globules, each indicating the position of a zoospore; *j–l*, in these sporangia the globules are slightly smaller and probably represent a more mature stage; *m–o*, dehiscence, part of the contents had been extruded into a vesicle; *p–s*, dehisced sporangia; *t*, specimen squashed while in stage such as *o* to show the thin wall which surrounds the extruded contents. × 1050.



Text-fig. 3. *Phlyctidium apophysatum*: *a*, two thalli united by a conjugation tube; at *x* is a swelling which later will become the resting spore; *b*, one thallus has produced a conjugation tube but no contact has been made with the neighbouring thallus which has grown into a minute sporangium; *c*–*k*, various stages in growth of the resting spore, note the diverse positions of it in the conjugation tube; *l*, mature resting spores of various shapes and sizes. × 1050.

wall and the content is dominated by a single large globule surrounded by many smaller ones. Mature resting spores vary in shape from spherical to subspherical or oblong. The resting spore may develop in a central position along the conjugation tube (Text-fig. 3*d*) or it may be practically adnate to one of the thalli (Text-fig. 3*f*, *g*, *j*). There does not appear to be any morphological distinction between the two conjugating thalli and no consistency in size. Sometimes both are more or less of equal size (Text-fig. 3*g*); at other times unequal. The specimen depicted in Text-fig. 3*f* may be abnormal as the very small thallus is situated in an area of the host cell devoid of chloroplast. Two interesting specimens were observed where a thallus has developed a conjugation tube but had not made contact with a second thallus. In that in Text-fig. 3*b* the thallus to which the conjugation tube was directed had grown into a minute sporangium. Again, Text-fig. 2*e* is of a thallus which presumably possessed sexual potentialities but for some reason unknown finally developed into a zoospore-producing thallus. Other instances of a similar behaviour are recorded for *Zygorhizidium willei* Löwenthal, *Rhizophydium contractophilum* Canter and *R. columnaris* Canter.

As no very early stage in the contact of the two thalli associated with a resting spore was found the exact method of its formation remains unknown. It is probably formed as in *Polyphagus*, but the possibility cannot be overlooked that a process similar to that found in *Rhizidium windermerense* Canter (1950) is involved.

There is no doubt that the chytrid found at Tarn and 'Ellerbeck' relates to *Phlyctidium apophysatum*. However, if this description is compared with the original one differences are seen. Development is endo-exogenous, a fact which was not observed in the original material. Again two types of sporangia were seen in the original sample. First, large ones which contained 100 or more minute oval zoospores, and secondly, smaller ones with a few large globules. No dehiscence of the latter sporangia was seen but the content indicated a larger zoospore with a conspicuous globule. This would agree with the zoospore as described for the Tarn and 'Ellerbeck' material where only this type of sporangium has been found. It is now believed that the sporangia with minute zoospores from the Clay pond must have been infected by another chytrid, possibly *Rozella*. Unfortunately the specific diagnosis was based on these sporangia. In the light of these new studies it is not at all certain that this chytrid should remain in *Phlyctidium*. In its method of development it somewhat resembles *Phlyctochytrium unispinum* Paterson but no resting spores are known for this species. Indeed so little is known about resting spore formation in species of *Phlyctidium* and *Phlyctochytrium* that to transfer it to the latter genus would, I feel, still only represent a temporary resting place. It may well be that a new genus will be needed to accommodate inoperculate species with endo-exogenous development and an endobiotic resting spore. Thus I leave this chytrid as *Phlyctidium apophysatum* and emend the original diagnosis.

PHLYCTIDIUM APOPHYSATUM Canter emend.

Thallus monocentric, consisting of an epibiotic sporangium and an endobiotic prosporangium and rhizoid. Development is endo-exogenous. Prosporangium 1·5–10 μ broad × 1·8–7 μ high. Rhizoid unbranched, tubular or sac-like 3·5–24 μ long × 0·5–7 μ broad. Sporangium spherical 9–17 μ diam., or ovoid 5–24 μ high × 4–13 μ broad, containing from 2 to 40 zoospores. Zoospore 3·5 μ diam., with a large colourless globule; flagellum 21 μ long. After deliquescence of the apex, a part of the mature sporangial content emerges surrounded by a membrane continuous with the sporangium. The zoospores are liberated by the rupturing of this membrane. Wall of empty sporangium persistent. Resting spore endobiotic, spherical, subspherical or oblong 7–15 μ broad × 6–10 μ high. Wall thick colourless, content dominated by a single large globule 4–7 μ diam., formed by fusion of two, usually anisogamous thalli and located in any position along the length of the conjugation tube joining the thalli. Germination not observed.

Parasitic on *Mougeotia* sp., Clay Pond, Wray Castle, Lancs., the tarn at Tarn near Silloth, Cumberland, and pond at 'Ellerbeck', Ambleside, Westmorland.

My grateful thanks are due to Dr J. W. G. Lund for reading the manuscript. Acknowledgement is made to the Central Research Fund of the University of London, for the loan of a microscope with which this investigation was carried out.

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EXPLANATION OF PLATE 34

Phlyctidium apophysatum. × 1360

Fig. 1. Encysted zoospore and broad germ tube set at right angles to the long axis of the host cell.
Figs. 2, 3. Growth of prosporangium and tubular rhizoid.
Fig. 4. Wall of zoospore case greatly thickened, rhizoid now lies parallel to length of algal cell.
Figs. 5–8. Budding out and subsequent growth of sporangium through the original zoospore case.
Fig. 9. A mature sporangium.
Fig. 10. Dehisced sporangium, endobiotic system now unrecognizable.

(Fig. 8 from Clay Pond, the rest from Tarn)

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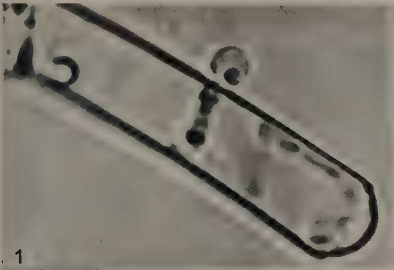
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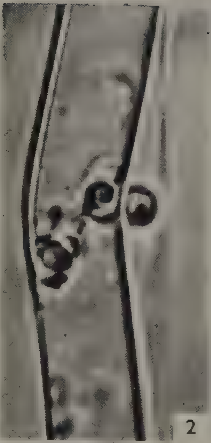
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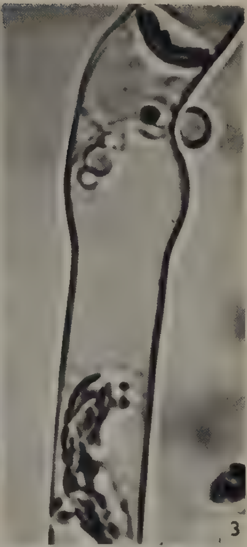
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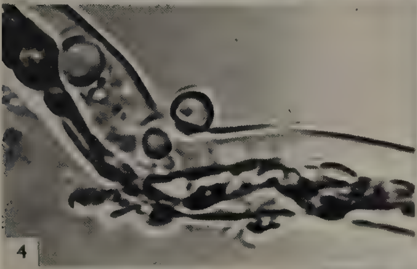
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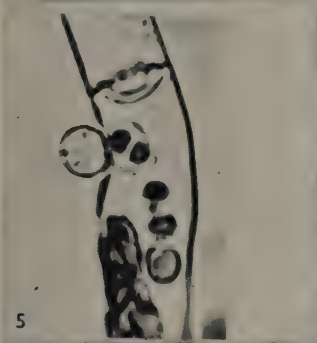
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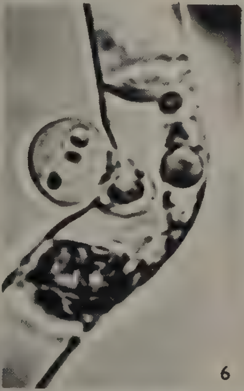
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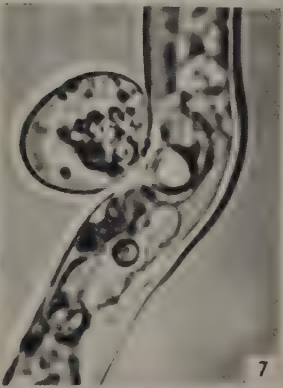
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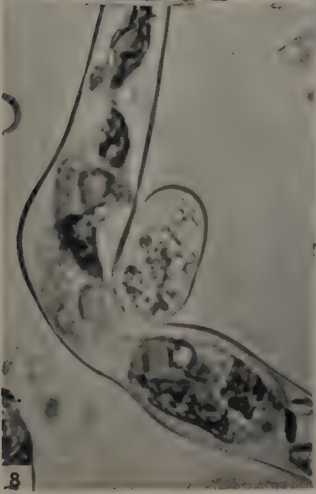
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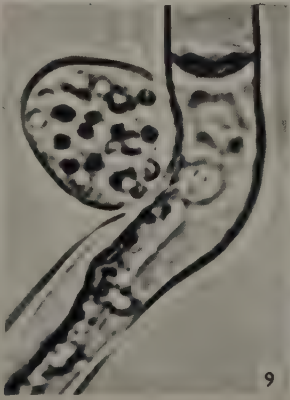
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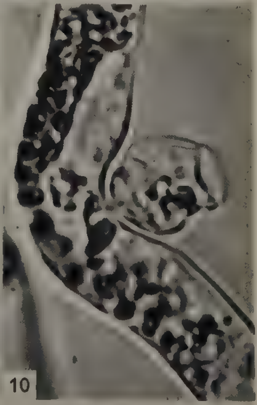
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Trans. Brit. mycol. Soc. **44** (4), 529–545 (1961).

FURTHER OBSERVATIONS ON AN ABNORMAL REACTION OF WHEAT TO LOOSE SMUT

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(With Plate 35 and 2 Text-figures)

A seedling wheat reaction to loose smut infection, characterized by a growth inhibition and leaf chlorosis, has been anatomically investigated. Loose smut has been established as the cause of the reaction, and increasing severity of symptoms has been correlated with the extent of mycelial distribution within the plumular bud. Although previously attributed to hypersensitivity, this type of host/parasite interaction does not fit the present accepted definition of a hypersensitive reaction and in this connexion the term ought not to be used.

Loose smut of wheat caused by the fungus *Ustilago nuda* (Jens.) Rostr. has generally been regarded as having little or no effect on the growth of the host plant until the ear is partially or completely replaced by a mass of black spores. The reaction of a wheat variety to the fungus may be divided into two basic categories, susceptible or resistant, according to whether or not smutted ears are produced. The susceptible reaction has recently been reinvestigated by Batts & Jeater (1958*a*), and resistant reactions were studied by a number of workers including Oort (1944), Popp (1951), Ohms & Bever (1955), and Batts & Jeater (1958*b*).

The first observations of an abnormal reaction of some seedlings grown from inoculated seed were recorded by Oort (1944, 1953). Combinations, involving a number of spring wheat varieties and six Dutch physiologic races, were grown in the glasshouse and normally susceptible and resistant types were observed, but in a number of combinations Oort noticed seedlings which developed abnormally. The most striking symptom was a severe inhibition of growth shown by a shortening of the first three leaves, which was often accompanied by chlorotic stripes and spots and malformations causing a leaf curl. These symptoms were seen clearly only when inoculated seedlings were grown in the glasshouse. The seedlings showing the symptoms often died in the two- to three-leaf stage, but some recovered, either by the development of side shoots, or by the main axis resuming growth and completing its development. The plants which recovered were dwarfed but produced small healthy ears in all except one or two varieties, by which small smutted ears were produced.

Oort had no direct evidence that the phenomenon was caused by loose smut. However, he assumed it was so because the reaction was highly specific to certain spring wheat/loose smut combinations, and also because a few combinations gave smutted ears on plants which recovered after showing the symptoms in the seedling stage. He ascribed the phenomenon to hypersensitivity and suggested that the normally compatible host-parasite relationship had been disturbed so that the plant was severely affected and, with few exceptions, the fungus failed to sporulate.

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Since 1944 a number of workers have referred to Oort's paper but only Kiraly & Lelley (1957) have made any further investigations. Using Hungarian winter wheats they observed a hyperergic host reaction (*sic*) which inhibited or killed the pathogen. The plants which survived appeared to be healthy. Kiraly & Lelley claimed that these plants had recovered from infection by a hypersensitive reaction. Mycelium was often found in the lower nodes of the stem, but never in the ear or highest node.

The absence of an adequate explanation of the seedling reaction has stimulated an anatomical investigation of this phenomenon, to determine directly whether loose smut was always the cause.

Oort's observations have been further studied. The term hypersensitivity should not, however, be used for the symptoms described by him, because the definition of hypersensitivity adopted by the British Mycological Society (1950) is 'Giving violent local reaction to attack by a pathogen, the prompt death of tissue round the points of entry preventing further spread of infection'. As will be seen later in this paper, the course of events within the infected wheat plant does not fit in with this definition. The relationship between fungus and host later described would appear to be unlike any other so far reported in plant pathology, and none of the established terms (British Mycological Society, 1950, 1953) can be used for this relationship.

In this paper, therefore, the term compatible is used for the reaction of a wheat plant to the fungus whereby the ear is replaced by smut spores, there being no symptoms visible in the seedling stage. For the reaction ascribed by Oort (1944) to hypersensitivity and examined in detail here, the term incompatibility is used. No new meaning is being read into these terms. To avoid the repetition of the terms compatible and incompatible, the terms normal and abnormal, respectively, are also sometimes used.

MATERIALS AND METHODS

For the anatomical investigation of this incompatible reaction, seeds of two spring wheat varieties, Little Club and Kota, each inoculated with Dutch loose smut races 1–7 as supplied by Prof. Oort, were grown in John Innes compost in the glasshouse. The combinations included those previously classified as normally susceptible and hypersensitive (Oort, 1944).

During the winter, good growing conditions were maintained in the glasshouse by an average temperature of approximately 60° F., and fluorescent lighting controlled to 20 hr. by an automatic time switch. The occasional use of a 1000 W. tungsten filament bulb locally increased light and temperature, which was sometimes desirable to maintain active sturdy growth of certain wheat varieties.

Oort (1944) found that shortening of the leaf was the primary criterion in identifying hypersensitivity. A standard method of leaf measurement was used here in all experiments. Only the leaf blade, from the ligule to the leaf tip, was measured. Where the first blade had incompletely emerged from the coleoptile, the length which extended beyond the coleoptile tip was recorded. Where subsequent leaf blades had not fully emerged, the length which extended beyond the ligule of the preceding leaf was recorded.

Previous workers have used the embryo test (Simmonds, 1946) to estimate infection. The purpose of the present investigation was to establish whether loose smut was the causal factor in each plant showing the unusual symptons, and so the determination of mycelium in each plant was essential. Thus the embryo testing of samples was not sufficiently accurate, and a more satisfactory method was used. In an infected embryo, mycelium is always present in the scutellum. Excision of this tissue from a seedling in the two-leaf stage does not harm the seedling and examination, then, of the scutellum provides direct evidence of infection. Each seedling was carefully dug up and the lower parts washed to remove soil from the pericarp, which was then removed with fine scissors and the scutellum carefully cut off with a razor. The seedling was then replanted and grown to maturity.

To determine the presence of infection, the scutellum was placed in *c.* 10% sodium hydroxide maintained at or near boiling point. After 2–3 min. treatment the softened tissues were washed in cold water for a few minutes, mounted on a slide and stained with cotton blue in lactophenol. Gentle pressure on the coverslip with a needle, sometimes assisted by warming the slide, spread the tissues, which were then examined for infection by hand sections, Reichert freezing microtome sections, or as dissected pieces of tissue.

The apical growing point and very young leaf initials required no softening prior to mounting, and so, after dissecting out with fine needles, the tissue was mounted in cotton blue in lactophenol. Sometimes mycelium was obvious but the slide usually required warming to clear the tissue partly. Often, however, mycelium was only observed after very carefully allowing the mountant to boil. This completely cleared the tissue and assisted the staining of the hyphal walls.

Other tissues such as leaf tips and young stems were soaked in 95–100% ethanol to extract the chlorophyll, gently boiled in 10% sodium hydroxide solution for 10–15 min., washed in water to remove excess sodium hydroxide and mounted.

Before sowing, the seeds were surface sterilized for 20 min. in 30% sodium hypochlorite solution, washed thoroughly with several changes of sterile water, and imbibed for 4–5 hr. at room temperature, to ensure even germination.

EXPERIMENTAL WORK

Preliminary experiments

Oort (private communication) suggested that Little Club would show the hypersensitive symptoms more clearly than Kota, and so, in preliminary experiments, this variety was used to attempt to obtain the symptoms described by Oort (1944). Abnormalities of germination and early development were observed. Coleoptiles were often very short and quite insufficient to provide a guiding tube through which the epicotyl could pass to the surface. Consequently young leaves grew in various directions below the surface. Some coleoptiles seemed to respond positively to geotropic stimuli. Only rarely were these abnormal seedlings infected by loose smut, and, if replanted so that the distorted epicotyls were above soil level, they recovered to produce normal healthy plants. Thus Little Club appeared to have an inherent abnormality completely dissociated from loose smut infection, although a few abnormal seedlings had loose smut mycelium permeating the meristematic tissues of the growing point. Further preliminary experiments using Kota revealed seedlings showing symptoms similar to those described by Oort (1944), and subsequent detailed investigations were confined to this variety.

To investigate the distribution of mycelium within the germinating seed, a few seeds of Kota inoculated with race 1 were germinated in vermiculite until the coleoptiles had developed (approximately 1·5 cm.). The seedlings were then sectioned and stained with cotton blue. Pl. 35, fig. 1, indicates the distribution of the fungus in an infected young crown. The apical growing point was just initiating the fourth leaf and was partly enclosed by the third-leaf initial, which had been permeated by the fungus. Fragmented hyphae were also observed in the extending second leaf. The apical growing point and the tissues below it were heavily infected by loose smut mycelium. In some embryos the fungus had reached the apical growing point and penetrated leaf initials during the development and ripening of the seed, while it was still within the ear.

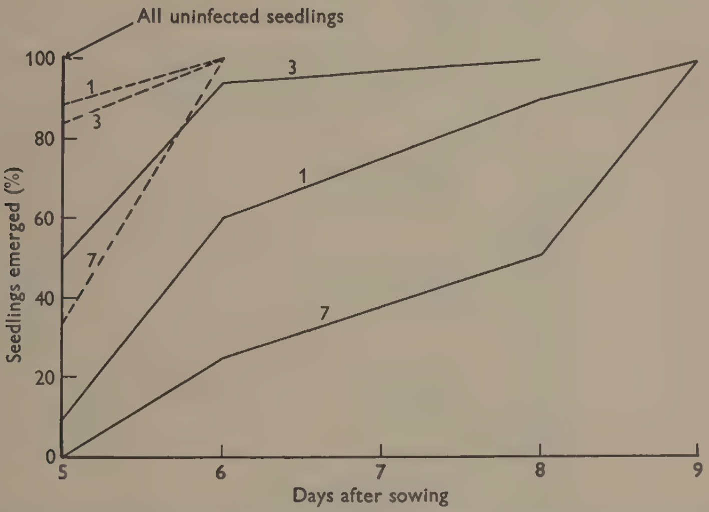
Detailed investigation of the incompatible reaction of Kota to three Dutch races of loose smut

Experiment 1. Forty seeds of Kota inoculated with Dutch races 1, 3 and 7 were grown in the glasshouse. Seedling emergence was recorded daily and leaf lengths were measured after 8, 12 and 15 days. After 16 days the seedlings inoculated with races 1 and 3 were dug up and preserved in 95 % ethanol. The seedlings inoculated with race 7 which showed incompatibility symptoms were similarly preserved while the scutella of those not showing such symptoms were removed and the plants grown to maturity. The length of the third internode of all seedlings was recorded and the distribution of loose smut mycelium in the preserved seedlings was subsequently investigated anatomically.

In each wheat/loose smut combination, seedlings were observed which clearly exhibited the two basic inseparable symptoms of the incompatible reaction: an inhibition of growth, shown most clearly by a reduction in leaf length, and chlorotic striping and spotting of the leaf blades. The scutellum of each seedling showing these symptoms was heavily infected with loose smut mycelium. The growth inhibition was noticeable during and after emergence. The seedlings which showed these symptoms emerged more slowly (Text-fig. 1), but all seedlings emerged in the normal way through the coleoptile, which always reached the soil surface before the first leaf broke through its tip.

The basic symptoms were usually already quite clear in the one-leaf stage. The normal seedlings had a long leaf, with the blade completely emerged from the coleoptile and a part of its sheath showing above the coleoptile tip. The blade was evenly green and regularly shaped. In contrast, the seedlings showing incompatibility symptoms usually had a distinctly darker green leaf, small in size, and often only partly emerged from the coleoptile so that its sheath was not visible. Chlorotic spots and longitudinal streaks were most clearly seen if the leaf was viewed against the light. The darker green colour of the leaves may have been due to the shorter leaves having a greater concentration of chloroplasts per unit leaf area because of reduced cell extension.

In normal plants when the second leaf emerged, the blade was again



Text-fig. 1. The comparative emergence rates of Kota seedlings inoculated with Dutch races 1, 3 and 7 grouped according to the presence of incompatibility symptoms (——), the presence of scutellar infection without incompatibility symptoms (– – – –), and the complete absence of infection.

extruded so that its sheath was visible beyond the ligule of the first leaf. In the abnormal plants, the second leaf emergence was incomplete and, in comparison with normal seedlings, growth inhibition was even more pronounced. At this stage the third internode normally elongates to bring the crown to the surface, and the growth inhibition of abnormal plants was also reflected in this aspect of development (Table 1).

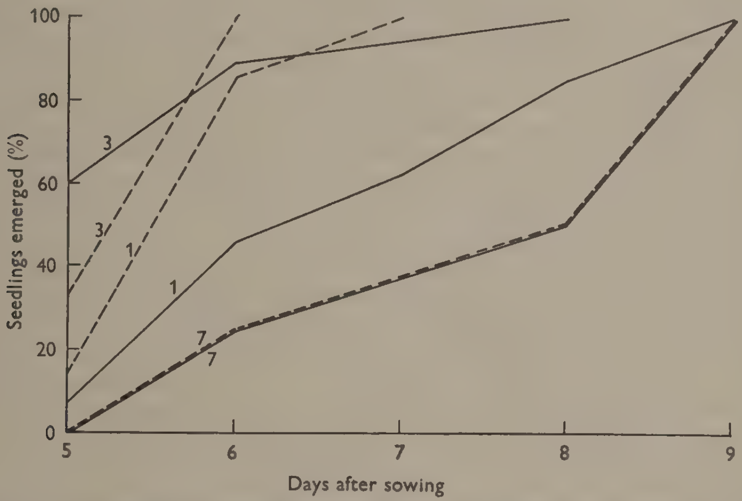
By the time normal plants had produced three leaves, there was a clear variation in the degree of incompatibility symptoms shown by the others. A few seedlings had only produced one small dark green chlorotic leaf, while others had two such leaves. A number of the latter seedlings showed signs of recovery. The most recently emerging leaf was a more normal shade of green, little or no chlorosis was observed and it subsequently grew longer than the preceding leaves; at this time the seedlings were dug up because preliminary experiments showed that only young seedlings were suitable for anatomical investigations, before other fungi, including *Fusarium* spp., gained entry to the weakened seedling.

Table 1. *Effect of infection by three Dutch races of* Ustilago nuda *on third internode length and on total leaf length of Kota wheat seedlings*

	Dutch race of *U. nuda*	No. of Plants	No. of Leaves after 15 days	Mean length of third internode (cm.)	As % that of uninfected plants	Mean total leaf length (cm.) Days after sowing 8	12	15	As % that of uninfected plants Days after sowing 8	12	15
Uninfected	(1+3+7)	37	3	2·7	100	20·6	40·1	52·4	100	100	100
No incompatibility symptoms. Mycelium confined to scutellum	1	9	3	2·3	85	16·6	36·2	47·3	81	90	90
	3	17	3	2·0	74	13·7	32·3	43·9	67	81	84
	7	3	3	3·3	100	14·3	33·0	43·3	69	82	83
Slight incompatibility symptoms. Partial crown infection	1	7	3	2·5	93	7·9	18·3	23·5	38	46	45
	3	6	3	2·1	78	8·1	16·3	23·6	39	41	45
	7	4	0–3	0·2	7	2·3	4·4	7·8	11	11	15
Severe incompatibility symptoms. Apical growing point infected	1	13	1–2	0·8	30	3·0	5·9	6·4	15	15	12
	3	10	1–2	0·9	33	6·8	9·6	9·6	33	24	18
	7	4	0–2	1·0	37	2·1	4·4	6·1	10	11	12

Other features sometimes associated with the basic symptoms of the incompatible reaction were a serrated leaf edge, splitting of the leaf sheath, spiral twisting of the leaf, and a curvature of the blade in its flat plane, often with a chlorotic edge to the inside of the curve.

Although abnormal seedlings usually had a fairly good primary root system, examination of the epicotyl sometimes revealed severe abnormalities. Cancerous proliferations of uninfected parenchymatous tissue were found near the growing-point region. Abnormal extension of internodes and tissues around the apical growing point resulted in the gross distortion of the growing-point region.



Text-fig. 2. The comparative emergence rates of abnormal Kota seedlings infected with Dutch races 1, 3 and 7 grouped according to partial crown infection (– – – –), or apical growing-point infection (——).

On the basis of the distribution of loose smut mycelium in the epicotyl, anatomical investigation revealed that all seedlings with infected scutella could be divided into three groups. The first group consisted of apparently normal seedlings, having no leaf chlorosis, although there was a slight growth inhibition; in all these plants the fungus was confined to the scutellum, and the amount of mycelium there was considerable. However, as a group, the total leaf length was significantly less than in the completely uninfected plants (Table 1). The third internode was also slightly shorter. With each race of loose smut, after about 2 weeks, there was a clear tendency for seedlings to recover from the slight early growth inhibition. All the plants inoculated with race 7 which were grown to maturity (some of which had scutellar infection) produced normal uninfected ears, which set seed.

The second and third groups were composed of abnormal seedlings only. The abnormal seedlings of each combination emerged more slowly than those with infection confined to the scutellum, or those which were completely uninfected (Text-fig. 1). There were also distinct emergence rates for abnormal seedlings infected with races 1 and 3 according to whether the fungus only partly invaded the crown, or had reached the apical growing point (Text-fig. 2).

The second group consisted of plants in which mycelium had entered the crown but had not reached the apical growing point. These seedlings were mostly those with the least severe symptoms. All the seedlings showing a tendency to recover were in this group, but occasionally only partial infection of the crown was associated with quite severe symptoms, particularly with race 7, and so these seedlings were also included. As a group, these seedlings were less than half the size of the uninfected seedlings (Table 1). There was a consistent tendency to recover, but with race 7 the plants were very stunted. The severe effect of race 7 was reflected by the almost complete inhibition of third-internode extension. With races 1 and 3 there was a slight reduction in the length of this internode. The distribution of the fungus in the partially infected crown was mainly intercellular and the hyphae had not penetrated further than the third node (associated with the base of the third leaf). Infection was often confined to the crown node (associated with the first leaf). According to the extent of infection within the crown, so mycelium was often found at the tips of the first, second, or third leaves. This was because during embryo development within maturing seeds in the inoculated ear, the fungus only partly entered the plumular bud, permeating the basal nodes and some of their associated leaf initials, but failing to reach the apical growing point. When seeds with this type of infection were germinated, the mycelium which had permeated lower leaf initials was isolated at the tips of the expanding leaves. However, the fungus did not always penetrate each leaf initial. Thus the first and third leaf tips could be infected while the second was free from infection, as a result of the fungus by-passing the second leaf initial of the embryo. A reduction in the number of leaves above the apical growing point, as compared with uninfected seedlings, was correlated with partial crown infection; the apical growing point of a partially infected crown initiated leaves at a slower rate than usual. In one abnormal seedling with partial crown infection, a small group of mature spores was found embedded within the tissues near the second leaf tip. The only mycelium in this leaf was distal to these spores. This was the only occurrence of spores within a leaf of a plant showing symptoms of incompatibility.

The third group consisted of seedlings showing the most severe symptoms. This severe reaction was always associated with an infected apical growing point, i.e. complete infection of the crown, the fungus penetrating into the extreme meristematic tip of the stem apex. The seedlings infected in the apical growing point with races 1 and 3 emerged more slowly than those in the preceding group, in which the fungus only partly invaded the crown (Text-fig. 2). However, seedlings infected in the apical growing point with race 7 emerged at a rate similar to that of the preceding group. The third internode length was reduced by 60–70 % with each race (Table 1). The total leaf length reflected the severe growth inhibition and there was no tendency for the seedlings with infected apical growing points to revover from the severe incompatibility symptoms. Mycelium was usually found in the leaf tips (Pl. 35, fig. 2), but axillary buds were not usually infected. Little or no further activity of the apical growing point had occurred since germination so that only three, or sometimes four, leaves above this meristem were present. It was not possible to correlate the severity of growth inhibition with the density of apical growing-point infection. The quantity of mycelium varied considerably; so did the readiness with which it was stained by cotton blue, possibly suggesting that there was variation in the thickness of the fungal wall.

The results indicated that infection of the crown by race 7 was associated with the most severe reaction. Races 1 and 3 were not so clearly distinguished by their effect, but crown infection by race 1 produced a slightly more severe reaction than did infection by race 3.

Experiment 2. The preceding experiment, on plants approximately 2 weeks old, established a direct correlation between loose smut infection of the crown and incompatibility symptoms. No necrosis in seedling tissues had been observed and it seemed probable that anatomical investigation would be just as practicable after 3–4 weeks. The seedlings in Exp. 1 were also too young for tiller development, or for substantial main axis recovery. Therefore a further twenty seeds of each combination were grown to provide older material for the investigation of recovery and to confirm the previous experiment. Leaf lengths were measured after 11 and 25 days. On the twenty-sixth day, the seedlings showing incompatibility symptoms were preserved for investigation. The other seedlings which did not show symptoms of incompatibility were also dug up. The scutella were removed and the seedlings replanted and grown to maturity.

Only one seedling, inoculated with race 7, had not emerged by 11 days after sowing. The coleoptile was 1·0 cm. long and the first leaf extended 0·5 cm. beyond. A tiny second leaf enclosed an infected apical growing point and mycelium was observed at the base of each leaf. There was no external necrosis, nor infection by other fungi. It appeared that this seedling was reacting severely to apical growing-point infection.

The grouping of seedlings showing incompatibility symptoms, according to the distribution of mycelium within the crown, was confirmed. Seedlings of each group were observed in each combination.

The basic incompatibility symptoms were clear in all the seedlings examined. In addition to the growth inhibition and chlorosis, leaf curling was observed and, by comparison with the normal seedlings, the incomplete emergence of some leaves from the coleoptile or from the preceding leaf was quite marked.

The leaf lengths (Table 2) illustrate the growth inhibition correlated with the three groups of seedling infection. These groups are quite distinct in each combination. Comparing the two groups of infection within the seedlings showing incompatibility symptoms, in each combination the more severe reduction in size was where the apical growing point was infected. Seedlings infected with race 7 were smaller than those infected with race 1, which again were smaller than those infected with race 3. This was also shown by the number of leaves present on each group of seedlings infected with either race of smut fungus. Where infection was confined to the scutellum, there was only a slight reduction in leaf length. The tendency for seedlings with only scutellar infection to be slightly reduced in size at 11–25 days after sowing, relative to uninfected seedlings, was probably due (*a*) to the rapid growth of the uninfected plants, (*b*) to the length of any of their leaves being greater than the preceding leaf at this stage.

The infection groups were distinct within each combination. Also, within each group, infection by any of the three Dutch races still fell within a distinct range (Table 3).

Table 2. *Effect of infection by three Dutch races of* Ustilago nuda *on leaf length of Kota wheat seedlings*

				Mean total leaf length					
		No. of		All leaves				1st 3 leaves	
	Dutch race of *U. nuda*	Plants	Leaves after 25 days	11 days		25 days		25 days	
				(cm.)	%*	(cm.)	%*	(cm.)	%*
Uninfected	(1)	2	4	14·0	100	71·0	100	63·3	100
	(3)	5	4	18·1	100	81·9	100	66·0	100
	(7)	12	4	15·8	100	70·8	100	65·1	100
No incompatibility symptoms. Mycelium confined to scutellum	1	11	4	11·5	82	52·5	74	49·2	78
	3	10	4	16·3	90	73·6	90	63·2	96
	7	1	4	15·0	95	65·5	93	60·5	93
Slight incompatibility symptoms. Partial crown infection	1	3	4	8·3	59	28·8	41	27·3	43
	3	1	4	13·0	72	41·0	50	29·5	45
	7	2	3–4	5·0	32	23·0	33	21·5	33
Severe incompatibility symptoms. Apical growing point infected	1	4	2–3	6·5	46	14·6	21	14·6	23
	3	4	2–4	11·0	61	21·1	26	20·9	32
	7	4†	2	4·8	30	11·8	17	11·8	18

* Length expressed as % that of uninfected plants.
† Plus one non-emerged seedling.

Table 3. *Relationship in Kota wheat seedlings infected with Dutch races* 1, 3 *or* 7 *between presence of incompatibility symptoms, distribution of loose smut mycelium, and total length of first three leaves*

Incompatibility symptoms	Distribution of mycelium	Total length of 1st three leaves as % that of uninfected plants
None	Confined to scutellum	78–96
Slight	Partial crown infection	33–45
Severe	Within apical growing point	18–32

A few seedlings which showed incompatibility symptoms began to recover, either by the main axis continuing growth or by the development of a tiller. Anatomical investigation showed that, where the main axis was recovering, the apical growing point was not infected and there were six or seven leaves above this meristem. Infection had not passed beyond the fourth node of the crown. The latest (third or fourth) emerged leaf did not show the symptoms, and the plant seemed able to grow away from infection situated lower down the crown. None of these plants produced a tiller nor had any of the normal plants, which were later grown to maturity.

When a tiller was produced it usually developed from a bud in the first or second leaf axil. The seedlings with tillers were infected either in or at the base of the apical growing point. These tillers were usually uninfected and developed normally, contrasting with the dwarf, chlorotic main axis. However, one seedling with an infected, abnormal main axis also had an abnormal tiller, which was infected up to apical growing point, but other uninfected axillary buds were developing. It seemed probable that if this plant recovered further, it would be by one of these uninfected buds.

Thus, within the limits of biological variation, infection of the apical growing point was associated with the lowest number of leaves initiated by this meristem. Partial infection of the crown, though generally having an inhibitory effect on leaf initiation, was usually associated with a slightly higher number of leaves than where the apical growing point was infected. This indicated that the nearer the pathogen came to the apical growing point, the greater the inhibition of meristematic activity within this tissue.

The reduced activity of the apical growing point due to loose smut infection was associated with a tendency for the buds in lower leaf axils to develop earlier than usual. The normal plants grown to maturity did not produce tillers until much later. It is possible that apical dominance had been broken as a result of the reduced apical activity.

The abnormal seedlings had no internal or external necrosis. The presence of loose smut within the crown did not appear to be associated with any browning of the host tissue and no other fungi were observed growing on or within the tissues. There was thus no recognizable factor, which could have caused the symptoms exhibited by the host, other than the systemic infection by loose smut. The seedlings which had not shown any symptoms were grown to maturity. No smutted ears were produced and all the ears set seed.

Experiment 3. Exps. 1 and 2 had established a direct correlation between the incompatibility reaction and loose smut infection. A third experiment was planned to test whether the seedlings with the least severe symptoms would recover by the main axis, because the apical growing points were not infected. Also, would the most severely infected seedlings only recover by a tiller, because the apical growing point of the main axis was infected?

A further twenty seedlings, inoculated with race 1, were grown undisturbed to maturity, and plants showing symptoms in the two- to three-leaf stage were divided into two groups, according to the severity of the symptoms. Seven seedlings did not show any symptoms while six showed slight, and seven showed severe symptoms of incompatibility.

All the plants which did not show any symptoms developed by the main axis in the usual way to produce normal healthy ears, which set seed as did the abnormal seedlings which recovered.

The seedlings with slight symptoms all recovered by the continuation of the main axis, and produced normal healthy ears which set seed. At maturity the two tallest plants in the pot (measured from ear tip to soil level) were from this group. There was no significant difference at maturity between the average height of plants in this group and that of those which did not show symptoms. Thus there had been a complete recovery of this group, and at maturity there was no evidence that the seedlings had shown symptoms.

Three of the seven severely abnormal seedlings died in the two- to three-leaf stage. The four remaining seedlings recovered to give normal healthy ears which set seed. Three of these had recovered by a tiller, the main axis having died, and one recovered by the main axis. However, these four plants were the smallest in the pot at maturity, their average height being 61 % of that of plants that had shown no symptoms. The smallest plant of all, 50 % of the normal, was that which had recovered by the main axis.

The inference drawn from Exps. 1 and 2, that seedlings showing incompatibility symptoms could be divided into two groups, was thus confirmed by Exp. 3. The plants with the least severe symptoms recovered by the main axis, while recovery from severe symptoms was usually by the growth of an uninfected tiller. It would have been interesting to know whether, in Exp. 3, the smallest plant which recovered by the main axis from severe symptoms had ever been infected within the apical growing point, but it was experimentally impossible to test the apical growing point for infection and allow the plant to grow on by the main axis. However, from the preceding experiments it was thought probable that the apical growing point had not been infected. Although only slight symptoms were usually associated with partial crown infection, the smallest seedlings did not always have an infected apical growing point. Some seedlings reacted severely to partial crown infection, and it is reasonable to suppose that it would be possible for even the most severely affected seedling to recover from partial crown infection provided that the apical growing point was not infected. However, these plants had been grown under advantageous conditions, and there had not been the competition between plants as would have occurred in a field crop, where weakened plants recovering from incompatibility symptoms would not necessarily be expected to recover so effectively.

Discussion

The occurrence of the basic symptoms of incompatibility, a growth inhibition and leaf chlorosis, has been correlated with the distribution of the fungus within the host plant. Seedlings from each host/parasite combination studied could be divided into four distinct groups as follows:

1. Completely uninfected. Normal growth of the plants.
2. Mycelium confined to the scutellum. No incompatibility symptoms, but a slight growth inhibition in the early seedling stage which is not noticeable in later growth.
3. Partial infection of the crown but the apical growing point is not infected. Slight incompatibility symptoms but the seedling may recover by the continuation of the main axis.
4. Apical growing-point infection. Severe incompatibility symptoms. Some seedlings die while others recover by an uninfected tiller.

Therefore, the symptoms first ascribed by Oort (1944) to hypersensitivity may now be regarded as directly related to crown infection by certain races of the loose smut fungus.

Oort noticed that many plants showing the symptoms died in the two- or three-leaf stage. From the present investigation this has been found to occur only when the apical growing point was infected. Oort also noticed that some abnormal seedlings recovered, either by a tiller or by the main axis. This is now more precisely attributed to the location of infection. Abnormal seedlings recovered by the main axis only if the apical growing point was not infected, and recovery by a tiller usually occurred only when there was complete infection of the crown, the fungus having reached the apical growing point. Axillary buds were usually uninfected. If an infected bud was stimulated to develop, because the apical growing point of the main axis was infected, it produced an abnormal tiller, and any further recovery would be by the development of an uninfected bud. The pathogen appeared to be attracted to the growing-point region of the main axis and by-passed most of the axillary buds, which thus escaped infection, possibly by chance. However, this may be due to a submaximal growth rate of the fungus within the tissues of a host showing these symptoms. Even in the normal compatible reaction, the degree of compatibility between host and parasite, as shown by the percentage smutting after inoculation, is closely reflected by the proportion of smutted to healthy tillers on the infected plants (Oort, 1947; Batts, 1955*b*). This is presumably related to a varying growth rate of the pathogen, which determines the proportion of axillary buds that will be permeated.

Oort (1944) distinguished the following four groups of seedlings within any inoculated 'hypersensitive' combination, on the basis of total length of the first three leaves:

Group		Description		Total length of first three leaves as % that of normal plants
a	'Hypersensitive' symptoms	Plants die as seedlings		0–28
b		Dwarf plants 25–75 %	of normal plant height at maturity	29–56
c		Small plants 75–100 %		57–84
d		Normal plants		85–100

The four groups of seedlings here described for Kota fit fairly closely with Oort's groups *a–d*. Groups *a* and *b* are somewhat similar to the severely and slightly 'hypersensitive' plants with complete and partial crown infection, respectively. However, from the present investigation, it seems that some seedlings showing severe symptoms, which according to Oort's classification on the basis of leaf length would be destined to die as seedlings, may recover by a tiller. Although the seedlings which die after showing symptoms are usually those with the most severe growth inhibition, it does not necessarily follow that, because the length of their first three leaves is less than 28 % of the normal, they will not recover.

Group *c*, in the upper part of its range, would include many of the seedlings which had infection confined to the scutellum in the present investigation. In this group there was a distinct effect of scutellar infection on early growth, which showed itself only as a slight reduction in leaf length.

Group *d* is equivalent to uninfected plants. To distinguish between groups *c* and *d*, the scutella must be examined. The presence of mycelium in the plumular bud directly or indirectly induces growth inhibition and leaf chlorosis. That this is a direct effect of the presence of the fungus is shown by the increasingly severe reaction that results according to whether the pathogen is (1) confined to the scutellum (2) partly permeates the plumular bud (3) reaches the apical growing point. Such marked differences in reaction are remarkable, depending as they do on fungal distribution within a few mm. of plant tissue. The presence of the fungus appears to inhibit cell division and probably also reduces cell extension.

Where the fungus reaches the apical growing point of the embryo, it appears to be purely circumstantial that the subsequent severe reaction to infection eventually constitutes a type of resistance. It is suggested that in this type of infection the presence of the fungus within the apical meristem inhibits growth, possibly by the production of some inhibitory metabolite. There was no evidence that the plant was directly opposing the presence of the fungus. Naturally, the main axis did not develop further and sporulation of the fungus was arrested because no ear was produced. Only once in a seedling showing symptoms of incompatibility were spores found in the tissues of an infected leaf tip. Where the fungus only partially penetrated the crown (or plumular bud) the reaction was less severe, and, although inhibited during early growth, the apex could eventually grow away to produce a normal sized plant in the glasshouse. It may be that, in such embryos, failure of the fungus to reach the apical growing point was due to some host reaction.

Where infection was confined to the scutellum there was a slight inhibitory effect on the length of the first two or three leaves, but no significant effect on the apex was observed. Plants with this type of infection did not show any leaf chlorosis. Thus it seems probable that the growth inhibitor is not readily diffusible through the embryo from the site of fungal infection. Even heavy infection confined to the scutellum had only a slight inhibitory effect on Kota seedlings.

Kiraly & Lelley (1957) investigated a seedling reaction of two Hungarian winter wheat varieties to loose smut infection. They stated that a hyperergic host reaction (Gäumann, 1950) had inhibited or killed the pathogen. Thus, the disease (smutted ears) failed to break out even if the host survived. They had not investigated embryos or young seedlings. Apparently, the seed was field-sown, and at ear emergence the nodes of the culms were investigated. In plants which never showed 'hypersensitive' symptoms, mycelium was not found in any of the nodes, but, in plants which showed 'hypersensitivity', mycelium was often found in the lower nodes of the culm. This was regarded as proof that these plants had recovered (presumably by the main axis) by a hyperergic seedling reaction. It is significant that mycelium was not found in lower nodes of all the 'hypersensitive' plants which had recovered. A seedling and embryo investigation of these combinations would have been interesting.

By definition (British Mycological Society, 1950), the essential feature of a hypersensitive reaction is a rapid necrotic isolation of infection. Because necrosis of infected tissues is not a feature of the seedling wheat reaction to loose smut infection, the reaction may not strictly be regarded as one of hypersensitivity. Further, there is no reaction to initial infection of the embryo, i.e. when mycelium first enters the scutellum, nor any subsequent necrotic isolation preventing further spread into the plumular bud. Müller (1959), however, suggests that meristematic tissue probably has the least tendency to produce a hypersensitive reaction.

An embryo, which, on germination, shows growth inhibition and leaf chlorosis, has a fungal distribution similar to that already well demonstrated for seedlings which, after normal early growth, produce a smutted ear (Batts, 1955*a*). Development of the embryo is not affected by loose smut mycelium growing within it during the maturation of the seed. Batts (1955*a*) found that, in susceptible (compatible) combinations, mycelium did not reach the embryo until about a month after inoculation, by which time embryo development was well advanced. From the present investigation of Kota, the fungus would not be expected to affect the apical growing point until the mycelium had entered the plumular bud, and by this time the development of the embryonic structures would be almost complete.

The absence of internal necrosis during manifestation of symptoms indicated that subsequent death of the main axis, when the apical growing point was infected, was due to secondary infections (i.e. necrosis from the outside inwards). Secondary infections, due to *Fusarium* spp. etc., were often present in dying seedlings. It seems reasonable to suppose that a stunted, diseased seedling would be less able to resist other infections than one which was actively growing. Ohms & Bever (1955) similarly attributed their reported seedling wheat mortality mainly to other soil organisms following infection by loose smut.

Ohms & Bever (1955) investigated the reaction of two winter wheat varieties to loose smut infection. In Kawvale, infection was mostly confined to the scutellum and there was no seedling mortality, but infected seedlings had a reduced third-internode extension which was compensated by extension of the fourth internode. This brought the crown to soil level, which action in uninfected plants was accomplished entirely by the third internode. This reaction was most noticeable in the two- or three-leaf stage. In Wabash, infection was accompanied by a severe seedling mortality in the three- to five-leaf stage. Statistical analysis revealed that the seedlings which died were probably infected, and none recovered by the development of a tiller. The seedlings which did not die were mostly uninfected, though a few had growing-point infection. However, the exact meaning of the 'growing point' was not made clear. The mortality appeared to be mainly due to soil organisms, since in sterilized soil there was less mortality. This reaction was different from that described by Oort (1944) because there was no leaf chlorosis, and some of the plants which survived produced smutted ears. Ohms & Bever termed the reaction 'over-susceptible'. Thus, this reaction of Wabash was a false resistance to loose smut and was probably due to a reduced resistance to other infections, resulting in the death of most infected seedlings.

There would seem to be some defence mechanism which confines infection of most field-resistant varieties to the scutellum, or at least prevents it from reaching the plumular bud. Popp (1959) has confirmed the conclusion of Batts & Jeater (1958*b*) that growing-point tissue of resistant varieties is not infected either before or after germination. Different varieties had varying distribution of mycelium within the embryo, but the plumular bud was never infected. Some moderately susceptible (compatible) combinations had a few embryos without plumular bud infection, and the proportion of infected embryos which also had mycelium within the plumular bud varied according to the degree of adult plant susceptibility or resistance. Popp concluded that 'clearly, infection of plumular bud tissue of embryos is directly related to the development of smut in adult plants.' The present investigations, however, have shown that plumular bud infection does not necessarily result in a smutted adult plant. The seedling may react to infection by growth inhibition and chlorosis, and any recovery results in a healthy ear.

I wish to express my gratitude to the late Dr C. C. V. Batts for his active interest in this work, to Prof. A. J. P. Oort for supplying wheat seed infected with loose smut, and to Mr A. Horne for taking the photographs.

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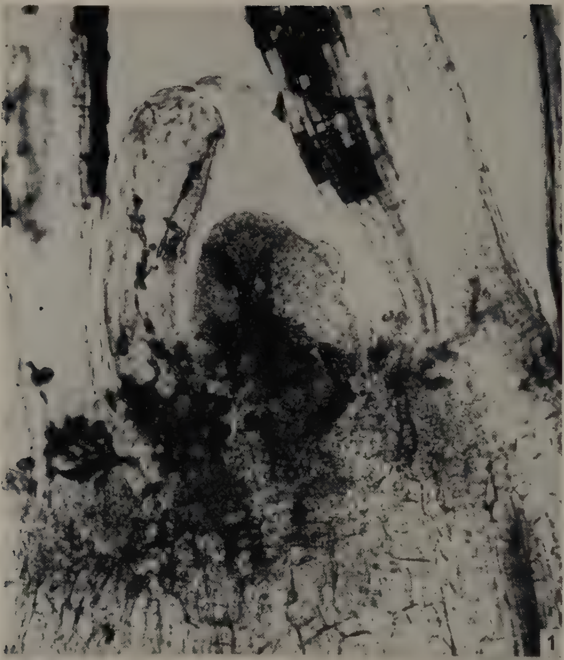
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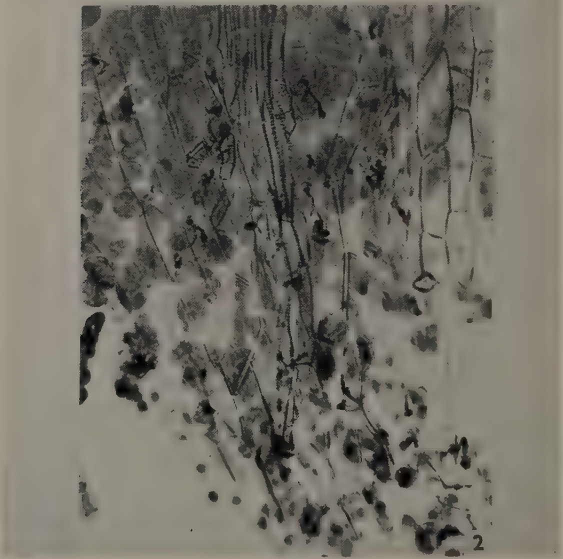
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EXPLANATION OF PLATE 35

Fig. 1. Longitudinal section of plumular bud of germinating Kota embryo, with mycelium within apical growing point and leaf initials. × 160.

Fig. 2. Systemic mycelium within leaf tip tissue of a Kota seedling, showing incompatibility symptoms. × 120.

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PERSISTENCE OF LOOSE SMUT INFECTION WITHIN THE GROWING POINT REGION OF SUSCEPTIBLE WHEAT VARIETIES

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When embryos of susceptible wheat varieties infected by *Ustilago nuda* are grown to maturity, the systemic mycelium within the embryonic plumule maintains its position in or near the apical meristem during host development.

The development of loose smut of wheat (*Ustilago nuda* (Jens.) Rostr.) has usually been described as 'keeping pace with the growing point'. However, Vanderwalle (1942) concluded that mycelium, present in the growing point of the embryo, was outdistanced by apical development during seedling growth and only later reached the developing ear as spikelet primordia differentiated. His conclusions were obtained from experiments in which he rooted nodes of wheat plants but did not ascertain whether the plants so treated were each infected. The problem has been re-investigated.

MATERIALS AND METHODS

Inoculated seeds of susceptible spring wheat/loose smut combinations were grown in John Innes compost in the glasshouse. When the third internode (the internode below the first foliage leaf insertion) had extended, in the two-leaf stage, the seedlings (of Bersee and Kota varieties) were dug up. The third internode was then severed with a razor 1 mm. below the crown node (the first node of the crown, associated with the first foliage leaf), and the excised epicotyls were placed in separate specimen tubes of distilled water under continuous fluorescent illumination until roots appeared. This usually occurred within a week. Meanwhile the scutellum of each seedling was removed from the remaining hypocotyl region and tested for the presence of loose smut mycelium (Mantle, 1961). Epicotyls from seedlings with infected scutella were then replanted in compost and grown to maturity. The same procedure was used on older Little Club seedlings excised in the three- and five-leaf stages at the internodes between the first and second (for three-leaf stage), or between the second and third (for five-leaf stage), nodes of the crown.

RESULTS

In the preliminary experiment with infected Bersee and Kota seedlings, which were rooted at the crown node, all the plants produced smutted ears.

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In the experiment with Little Club seedlings (Table 1), loose smut mycelium was easily recognized in the scutella of seedlings excised in the three-leaf stage, but at the five-leaf stage degeneration of the scutellum made it difficult to recognize infection. Thus the older seedlings were divided into two groups according to the certainty of infection. All the replanted epicotyls produced sturdy plants, and those with scutellar infection produced smutted ears.

Table 1. *Effect of excising infected wheat shoots on subsequent smutting*

Place of excision	Scutellar infection	Excised shoots	Smutted plants	Smutting (%)
Between 1st and 2nd nodes of crown	Present	17	17	100
Between 2nd and 3rd nodes of crown	Present	12	12	100
	Possibly present	13	11	85

DISCUSSION

Vanderwalle (1942) excised seedling epicotyls just below the crown node, rooted these shoots in warm moist soil, and at maturity observed a certain number of smutted ears (no numbers were given). Presumably he had taken a sample of seed, derived from previously inoculated ears, and by testing the embryos knew that a certain percentage were infected. A further sample was then used for the rooting of crown nodes, but because of low numbers it was not possible to apply statistical analysis. It was assumed that, in the sample used for the crown node rooting experiment, all the excised epicotyls which developed from infected embryos ultimately produced smutted ears. However, Vanderwalle concluded that there was a period when host development outstripped parasite development because, in a further experiment, where the epicotyls were excised between the first and second nodes of the crown, the few shoots which developed to maturity all produced healthy ears. Critical interpretation, therefore, of Vanderwalle's experiments would suggest that the mycelium always passed the crown node but that it lagged behind the next node.

In the present work, no reliance needed to be placed on statistical analysis. By ascertaining the presence or absence of infection in each embryo handled, the effect of epicotyl excision on each plant from an infected seed could be known. There was no evidence that excision at any point along the epicotyl affected the ultimate and inevitable sporulation of the systemic pathogen within the ear.

It is possible that the few plants which survived Vanderwalle's second experiment, and which formed healthy ears, were all derived from the uninfected fraction of the seed sample. Also, if as is usually found in susceptible combinations, the apical growing point of the germinating embryo is infected, then mycelium would already have passed three potential nodes of the plumular bud, which, by definition, later forms the crown. Thus, even if the pathogen made negligible progress during the first few days of host growth under glasshouse conditions, excision of the epicotyl between first and second nodes of the crown would fail to eliminate infection. Complete smutting of the ear following rooting of the second and third nodes of the crown has confirmed this deduction. Batts & Jeater (1958) expressed the opinion that, in highly susceptible combinations, loose smut persists in or very near the apical growing point throughout the host's development, and the present work brings forward experimental evidence in support of this view.

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EFFECTS OF DRYING ON THE VIABILITY OF GERMINATED AND GERMINATING CONIDIA OF *MONILINIA FRUCTICOLA* (WINT.) HONEY

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(With Plate 36 and 2 Text-figures)

Conidia of *Monilinia fructicola* were germinated, or brought almost to the point at which germ tubes would be produced, on dialysing membranes in contact with dilute peach juice. Membranes bearing these 'germinated' or 'germinating' spores were given a single exposure of 12, 24 or 72 hr. to drying atmospheres of *c.* 0, 15, 45, 75 or 90% relative humidity, and to temperatures of 8, 20, 25, or 30° C., and were then replaced on peach juice.

Germinated spores survived drying at 0 or 15% R.H. for at least 72 hr. at all temperatures. Those dried at 45–90% R.H. survived for shorter periods. At 30° C., the temperature at which injury developed most rapidly, no spores survived drying for 12 hr. Germinating spores were much less sensitive to drying, but severe injury developed when high temperatures were coupled with high humidities.

Drying is usually considered to be lethal to spores which have germinated, but have not penetrated the host (Doran, 1922; Duggar, 1901). Although this does not appear to be true for *Cercospora musae* (Calpouzos, 1955), *Aspergillus* (Duggar, 1901) or some of the powdery mildews (Yarwood, 1936), a great deal of circumstantial evidence indicates that it is true for many pathogenic fungi. However, very little quantitative information on survival of germinated spores is available. If only a small proportion of such spores survives a dry period, this may have a considerable effect on the epidemiology of the disease.

Conidia of *Monilinia fructicola* are extremely resistant to desiccation (Naqvi & Good, 1957). The objective of the present study was to determine to what extent this resistance was carried over into the period immediately preceding and succeeding germination. Conidia which had either been germinated, or had been held under conditions suitable for germination for a large part of the time required to produce germ tubes, were used as test material. These conidia were exposed for various periods to a wide range of drying atmospheres. The study was conducted purely as a laboratory investigation, and only the effects of one exposure to constant conditions are considered. An extension of the work to cover the fluctuating conditions, and repeated changes, met in nature has not yet been possible.

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MATERIALS AND METHODS

The fungus was isolated from a decaying peach. Several single-spore cultures were made from the original isolation, and one of these which showed good sporulation was used throughout the work. There was no change in its appearance or behaviour during the period of study.

To obtain spores which would germinate rapidly and as uniformly as possible, Petri dishes containing potato-dextrose agar (6% dextrose; Naqvi & Good, 1957) were inoculated at the centre and held in an incubator at 22° C. Under these conditions the colony spread about 7 mm./day. Spores produced chiefly on the third day previous could be obtained from the zone 20–30 mm. behind the advancing margin of the colony. Spores of this age were most suitable for rapid and consistent germination.

All observations were made on spores dusted onto cellulose membranes,* which had been boiled in three changes of distilled water to remove traces of glycerine or adhering material, and stored for as long as a week in distilled water in a refrigerator. No tests of the permeability of these membranes were made, but it is clear, from the excellence of the germination on them, that they are freely permeable to water, and at least to some of the dissolved material in the juice used as a germination medium.

Spores were lifted from the conidiophores with a fine camel-hair brush, and dusted directly onto a membrane held in one of the ways described below. For spore germination, the membranes were put in contact with a solution of 1 vol. peach juice to 9 vol. water. A large supply of juice, consisting of the filtrate from purée of fresh, ripe peaches was prepared at one time. This was put in small phials stored in a refrigerator at −18° C., and a fresh phial was used for each experiment. Dehydration of the membranes and spores was brought about at constant temperature in a closed system, in which the relative humidity was controlled by contact with a saturated solution of an appropriate salt (Hodgman, 1959).

For direct observation of the response of individual spores to drying and subsequent wetting, the membranes were mounted in a specially designed microscope stage, which permitted the application of the various conditions to the spores while keeping them under more or less constant observation. The temperature of the stage was held at 25° C., by a stream of water maintained at that temperature and pumped by a Bronwil Thermomix. Pairs of Fisher–Milligan gas-washing bottles fitted in series were used to give air of the desired humidity. Bottles filled with the saturated solutions were immersed in a water-bath at 25° C.

The special stage is illustrated in Pl. 36. It consists of a short aluminium cylinder with a chamber *P* in the centre. The bottom of this chamber is a plate of thin glass cemented into the cylinder. Two tubes, J_1 and J_2 lead into and out of *P*. A large groove *T* has inlet and outlet tubes W_1 and W_2 which provide for a rapid circulation of water through the main body of the cylinder. A lid, shown to the right of the figure, can be screwed to the cylinder by four milled nuts *H*. Two rubber '*O*' rings, shown below the main cylinder, may be fitted into groove *G* and onto the bevelled shoulder of the wall surrounding *P*. These rings provide an effective seal for the chambers of the apparatus. The lid is provided with a hole *S* round which a groove *O* is made to receive a glass coverslip. This is held in place by a ring *R*, and by clips *C*. Tubes A_1 and A_2 lead into and out of the space *S* below the coverslip. All surfaces in contact with either juice or water are coated with a thin film of 'Tygon' lacquer. The whole apparatus is attached to a square, 'Plexiglas' base-plate *B*, which fits firmly to the mechanical stage of a microscope.

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* 'U' Zephyr cellulose tubing supplied by the Visking Company, Lindsay, Ontario.

In operation, a membrane was placed over *P*, and the '*O*' rings dropped into position. The lid was applied and screwed down. As the lid was seated, the smaller '*O*' ring was pushed downward and outward along the bevelled edge of *P*, stretching the membrane taut. The spores were dusted on the membrane and the coverglass clipped into position. A supply of dilute peach juice was run in through J_1 until *P* was filled. Water was circulated through *T*, and air, previously passed through two of the Fisher–Milligan gas bottles to give *c*. 75 % R.H., was led through the space above the membrane at a rate of 25 ml./min. The spores were observed through a microscope fitted with an 8 mm. objective.

To subject the spores to drying conditions, the supply of juice was cut off and the centre well drained through J_2. Air of the desired humidity was then passed through the *J* and *A* tubes at the rate of 50 ml./min. so that both sides of the membrane were subjected to drying. Moisture was restored by filling *P* again with dilute juice.

When the behaviour of populations of spores (as distinct from that of individual spores) was being studied, the membranes were mounted between pieces of wax-impregnated fibre board, about 4 cm. square, in each of which a hole 2·5 cm. diam. had been cut. The pieces of board were held together with spring clips, and the assembly was laid on a van Tieghem cell cemented to a glass slide and filled with dilute peach juice. The membrane displaced the juice in the convex meniscus as it was fitted to the end of the van Tieghem cell, making complete contact with the surface of the liquid. It was held in place by an elastic band slipped over the glass slide and the pieces of fibre board.

The whole assembly described above was placed in a desiccator at 22° C. over a solution to give 75 % R.H. In such an atmosphere the membrane provided ample moisture for germination of the spores, but no troublesome condensation developed on the surfaces. For drying, the membranes were removed from the van Tieghem cells, and from the fibre-board holders, and free moisture was wiped from the under surface of the membranes, and from the surfaces of the boards. The membranes were fitted between the boards again, and these were placed in an atmosphere of the desired R.H. and temperature.

After drying, membranes were replaced on juice-filled van Tieghem cells and kept again in an atmosphere of 75 % R.H. Observations of the spores were made at intervals over a 24 hr. period. Each observation involved a count of germination in a sample of 300 spores. From the counts, curves of percentage germination against time could be drawn. In most cases, measurement of germ-tube length for 100 spores was also made.

OBSERVATIONS ON INDIVIDUAL GERMINATED SPORES

When membranes mounted on the stage shown in Pl. 36 were in continuous contact with the peach juice solution, most of the spores on them germinated within 4 hr., the germ tubes being one to four times the length of the spore. Drying of the germinated spores was brought about at this stage of development, by passing air through the apparatus on both sides of the membrane.

With air dried by passage through 'Drierite', a commercial laboratory desiccant prepared from anhydrous silica gel, the membranes became visibly dried and the germ tubes began to shrivel in 10–15 min. The same effects were seen when moister air was used, but they developed more slowly. With air of 90 % R.H., 40–60 min. were required to show the first definite evidence of drying. The spores were held dry for periods of up to 72 hr. before the peach juice was again brought into contact with the membrane.

Under all the conditions studied, dried spores and germ tubes became turgid within 1 min. of bringing the juice into contact with the membrane. Swelling was seen first in the body of the spore, from which it spread to the tips of the germ tubes. However, within 5 min. many of the germ tubes collapsed, and none of these collapsed germ tubes was seen to recover or show further growth. Those which did not collapse at this stage began to grow at a rate comparable to that of undried germ tubes. There was no evidence that the longer germ tubes were more susceptible to drying injury than were the shorter ones. Spores that survived drying at very low humidities took longer to recover than did those dried at high relative humidities. Those dried at 75 % R.H. showed growth after 1 hr., whereas those dried with air passed through Drierite required 3–4 hr. to recover to the same extent.

Although recovery was more rapid when spores were subjected to relatively mild conditions of drying, the percentage of spores surviving these conditions appeared to be appreciably lower than with the drying at very low humidities; some of the highest percentages of recovered spores were seen after drying with air passed through Drierite. Also, it was obvious that the spores retained their viability longer in very low humidities than in high ones.

Because the system of observing individual spores permitted observation of only a small number in any one experiment, the method described for studies of populations of spores was also used.

OBSERVATIONS ON POPULATIONS OF GERMINATED SPORES

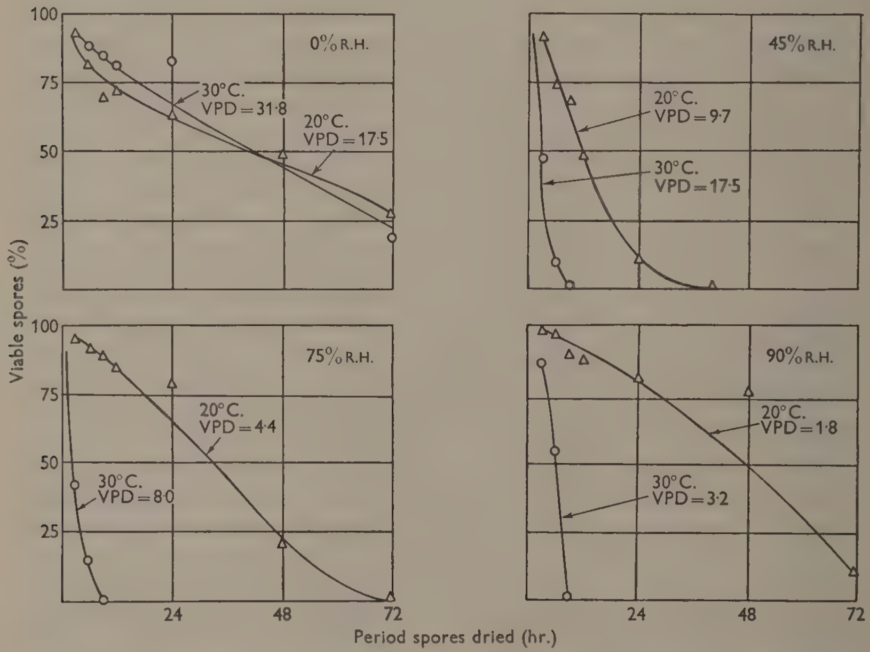
In all the experiments considered in this section, spores were dusted onto membranes held in the fibre-board holders, and counts were made of the percentage of germinated spores which remained viable after the drying period. The percentage of germinated spores was determined both before and after the drying period, so that allowance could be made for the few spores which had not yet germinated at the time of drying and which might otherwise have been included in the groups of spores surviving the dry

Table 1. *Percentages of germinated conidia of* Monilinia fructicola *surviving various drying treatments. Each value is a mean of at least three replications*

	Time of drying														
	12 hr.					24 hr.					72 hr.				
R.H. ...	0	15	45	75	90	0	15	45	75	90	0	15	45	75	90
8° C.	26±17	40±29	37±26	69±5	80±12	48±13	39±14	18±9	77±4	81±9	65±13	71±12	3±3	0	60±20
20° C.	49±21	49±25	30±25	46±14	80±4	65±7	50±15	20±13	56±21	62±21	25±6	26±10	0	0	3±2
25° C.	79±2	45±17	12±5	68±8	85±8	78±5	59±12	0	0	0	66±15	35±19	0	0	0
30° C.	55±17	42±17	0	0	0	85±5	35±20	3±2	0	0	15±2	19±11	0	0	0

period in the germinated state. Drying was done at 0, 15, 45, 75 and 90 % R.H. for periods of 12, 24 and 72 hr., and at temperatures of 8, 20, 25 and 30° C.

Two types of experiment were conducted. In the first type, several membranes were dusted with subsamples of one lot of spores and left in contact with peach juice for 4 hr., by which time almost all the spores had germinated. One membrane was placed under each of the humidity conditions. Only one temperature was used in each experiment and all membranes were removed at the same time for tests on spore survival. In the second type of experiment, spores from one sample were germinated on several membranes and all of these were placed in the same drying environment. Every few hours one membrane was removed, and viability of the spores tested. The first method permitted a comparison of the effect of the five different humidities on spore survival, whereas the second type gave a clearer picture of the rate at which injury developed. Results from both types of experiment are pooled in Table 1. Results from representative experiments of the second type are given in Text-fig. 1.



Text-fig. 1. Loss of viability of germinated conidia of *M. fructicola* in relation to time of drying at 20 and 30° C. and at relative humidities of 0, 45, 75 and 90 %. Vapour-pressure deficits (VPD) in mm. of mercury are given for each condition.

The test material proved disappointingly variable. However, the main trends were clear. Except at 30° C., a considerable proportion of germinated spores survived 12 hr. drying at all humidities. Longer periods were endured successfully when the spores were dried at low relative humidites.

High temperatures coupled with a relative humidity of 45 % or higher were damaging, but at 8 or 20° C. a considerable number of spores survived for 24 hr. at all humidities. Few survived for 72 hr., unless dried at very low relative humidities or at very low temperatures. The spores which were dried severely were much less sensitive to temperature or time of drying than were those dried under less severe conditions.

The rate at which the injury to the spores developed during the drying period is shown in Text-fig. 1. Except in a few instances, each point on the graphs is a mean of two determinations. The effects of temperature were very marked except under very dry conditions, in which temperature had little effect. At 30° C., all spores except those at 0 or 15 % R.H. were killed within 12 hr. At 20° C., however, spores survived as much as 48 hr. in all humidies except 45 %. Curves for 25° C. lay about midway between those for 20 and 30° C. A few spores dried at 25° C. and 0 % R.H. were viable after 6 weeks, and some dried at 8° C. and 0 % R.H. were able to continue to grow after 10 weeks.

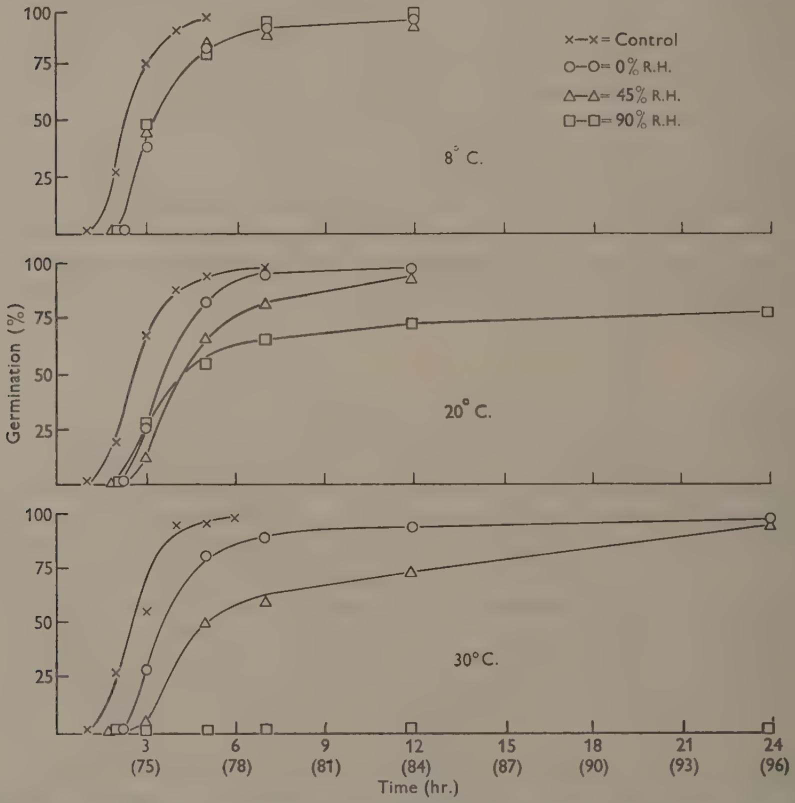
At both 20 and 30° C., spores were killed more quickly at 45 % R.H. than at any other humidity. This effect may also be seen in Table 1, in which the figures for surviving spores are consistently low in the columns under 45 % R.H. Although the material was highly variable, this effect deserves further attention.

Observations on germinating spores

Although spores which had germinated survived a surprising degree of drying, the germinated spore was clearly more susceptible to injury than was the ungerminated one. Whether this susceptibility was directly related to changes in the structure of the spore inherent in germination, or whether it was in part due to metabolic changes in preparation for the production of a germ tube was not known. To gain some insight into this question, spores were held for 1 hr. under germination conditions and then dried. This hour provided about three-quarters of the time required for the spores to begin to push out germ tubes. In each experiment subsamples of one lot of spores were dusted onto several membranes and placed over peach juice. One membrane was left on the juice as a control. The remainder were removed after 1 hr. and dried at one of the temperatures, one membrane being placed in each of the humidity conditions. After these 'almost germinated' spores had been dried for 72 hr., they were again placed under germination conditions and the germination-time curves determined. As before, 300 spores were observed for germination and 100 germ tubes were measured at each time of observation.

Results of the tests at 8, 20 and 30° C. are given as mean curves from three replications in the graphs of Text-fig. 2. Curves for the dried spores begin further from the origin than do those of the controls, because of the time required for the dried spores to become active again. Allowance should be made for this.

In all samples there was a decrease in the slope of the germination-time graph resulting from a delay in germination. This effect was barely noticeable at low temperatures and at low humidities, but became more marked as either of these factors increased. Humidity had little effect at low temperatures, almost all the spores surviving 72 hr. drying and germinating within 12 hr. At 20° C., the delay in germination was marked at 45 and 90 % R.H., and at 30° C. all the curves show a considerable decrease in slope. However, even samples held at 30° C. gave final percentages of germination which approximated to those of the controls, except in the case of spores dried at 90 % R.H. No germination occurred in 24 hr. in the samples stored at 30° C. and 90 % R.H. The behaviour of



Text-fig. 2. Germination-time curves for spores of *M. fructicola* exposed to germination conditions for 1 hr., then dried for 72 hr. at the temperatures and relative humidities shown, and finally returned to germination conditions. Figures in brackets include the drying period.

spores dried at 25° C. was almost identical with that of those dried at 30° C. Under all conditions tried, growth of the germ tubes was comparable to that of the controls.

The injury to 'almost germinated' spores developed a little less rapidly than did that to germinated spores. In samples dried for 12 hr. at 30° C. and 90 % R.H., germination was initiated after 12 hr. on peach juice and in three series 6–16 % spores had germinated in 24 hr. In samples dried for 24 hr. at 30° C. and 90 % R.H., no spores had germinated 24 hr. after their return to germination conditions. These figures may be compared with the complete kill of germinated spores in 9 hr. at 30° C. and 90% R.H.

The overall effects of drying were very much less drastic on the germinating spore than on the germinated one, being expressed more as a delay in germination than as a loss of viability. However, the relation between the injury and the environmental conditions inducing it were very similar for the two groups of spores. There was little injury unless a high temperature coincided with a relatively high humidity. At very low temperatures, humidity was of minor importance and at very low humidities the effects of temperature were much reduced.

Discussion

The injury to both germinated and germinating spores appeared to be more closely related to the manner of dehydration than to the extent. The severity of the dehydration conditions may be judged better from the vapour-pressure deficits than from the relative humidities. These are given in Text-fig. 1 for the range of conditions recorded there. There was no suggestion that it was dehydration *per se* which caused the injury. It appeared rather that the effect of drying on the cell was to produce abnormal metabolism, and that conditions which favoured rapid or prolonged abnormal activity resulted in severe damage or death. With rapid drying, the spores quickly shrivelled and presumably became inactive and resistant to injury, even at high temperatures or over prolonged periods. Experimental proof that the effect of drying is to produce abnormal metabolism would not be easy, since most metabolic studies are carried out under very moist conditions. It should be possible, however, to follow the evolution of carbon dioxide during dehydration, using spores grown on a labelled substrate.

It appears that germinated conidia of *M. fructicola* are capable of withstanding at least one prolonged period of drying and can still retain their ability to grow at a normal rate when suitable conditions return. However, in spite of the resistance to injury from drying under certain conditions, they are very susceptible to injury from even one moist period if this be followed by slow drying at a moderately high temperature. The interaction of temperature and humidity, both highly variable under field conditions, was very marked in this study. This makes it difficult to interpret the data presented here in terms of events in the field. Moreover, the conidia were subjected to a very abrupt rehydration in these experiments. That this can happen in rainy weather in the field cannot be doubted, but it is probable that a more gradual rehydration will be more frequent. Studies of the influence of rehydration rate, of the effects of several alternate wet and dry periods, and of the effects of drying at higher humidities common in the field, are needed.

It seems likely that the role of the spore dried after it has germinated, but before it has penetrated host tissue, will be found to be more important from the standpoint of plant infection than has been thought. Calpouzos (1955, 1961) has made observations on the conidia of *Cercospora musae* which indicate that these spores rarely penetrate during a single wet period. The germ tubes make some growth each night and are quiescent each day until, after 5–7 days, penetration of the leaf occurs. During this period, the germinated spores have been subjected several times to periods of high temperature and to at least some direct sunlight. Other observations such as those of Duggar (1901) also indicate that there may be considerable durability of some germinated spores. The whole problem is worthy of more study, though, as with most features of spore physiology, uniformity of behaviour among pathogens is not to be expected.

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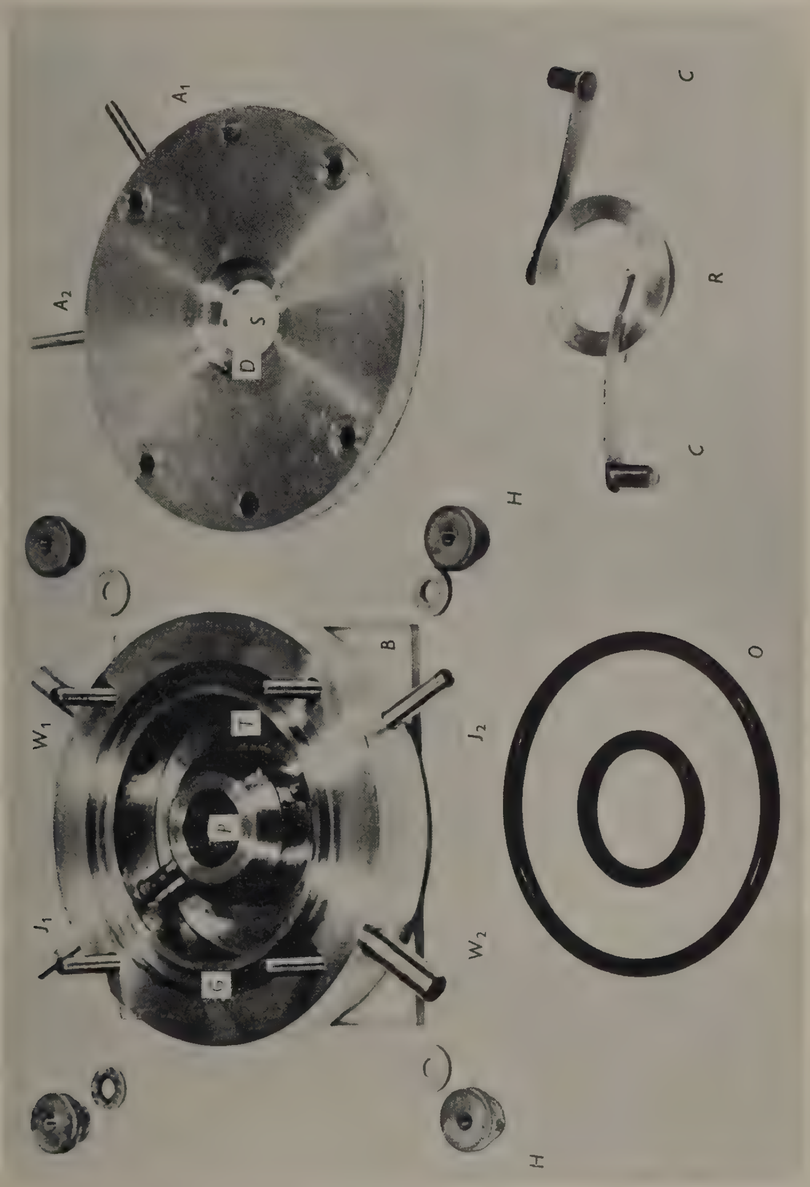
EXPLANATION OF PLATE 36

Showing the special microscope stage in which conidia of *Monilinia fructicola* were subjected to various environmental conditions and subsequently tested for viability.

(*Accepted for publication* 10 *February* 1961)



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Trans. Brit. mycol. Soc. **44** (4), 559–564 (1961).

THE *MOLLISIA* PERFECT STATE OF *ANGUILLOSPORA CRASSA*

BY JOHN WEBSTER

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(With 2 Text-figures)

Fructifications of a *Mollisia* appeared in 9-month-old multispore isolates of *Anguillospora crassa*. Isolates from ascospores confirmed that the two states are genetically identical.

In November 1959 a decorticated twig bearing noticeable mycelium, collected from the Limb Brook at Whirlow Brook Park, Sheffield, bore a vigorous growth of *A. crassa* Ingold (Ingold, 1958). The conidia were used to prepare a series of cultures and a number of single-spore and multispore isolates were established on stream-water malt extract agar (2 % malt extract agar made up in stream water) and left to grow at room temperature in a glass-sided cupboard on a north-facing window-sill. The fungus grew slowly, forming a dark grey felt with a white margin. When one of the multispore isolates was examined on 17 May 1960 three types of structure were observed:

(i) Dark swollen intercalary or terminal barrel-shaped or oval chlamydospores, 6–15 × 4–9 μ, borne singly or in small groups (Fig. 1B).

(ii) Small globose hyaline sticky phialospores (microconidia) borne in heads from clusters of phialides arranged in a penicillate manner at the tip of sparingly branched conidiophores. The phialides measured 6–8 × 1·5–2 μ and the phialospores 1·5–2 μ (Fig. 1A).

(iii) Coiled hyaline hyphae resembling ascogonia but without obvious trichogynes (Fig. 1C).

The presence of microconidia and presumed ascogonia suggested that some spermatization process might be necessary to procure further development. The culture, in a 6 cm. Petri dish, was flooded with sterile water and kept as before in the window cupboard. On 24 June 1960, about 5 weeks after flooding, six small grey apothecia with white disks of the *Mollisia* type were seen.

To ensure that the *Mollisia* had not entered the cultures as a contaminant with the original conidial inoculum of *A. crassa* a series of single and multi-ascospore isolates on slopes of stream-water malt extract agar in 6 × 1 in. boiling tubes were maintained in the glass window cupboard; for comparison a few slopes were inoculated at the same time from one of the original single conidial isolates of *A. crassa*. The two series of cultures were similar morphologically, though the conidial isolates grew rather more rapidly. Both cultures bore a dark greyish-green aerial mycelium with paler hyphae at the margin. When 1-month-old cultures were cut up and placed in water which was vigorously aerated with a stream of com-

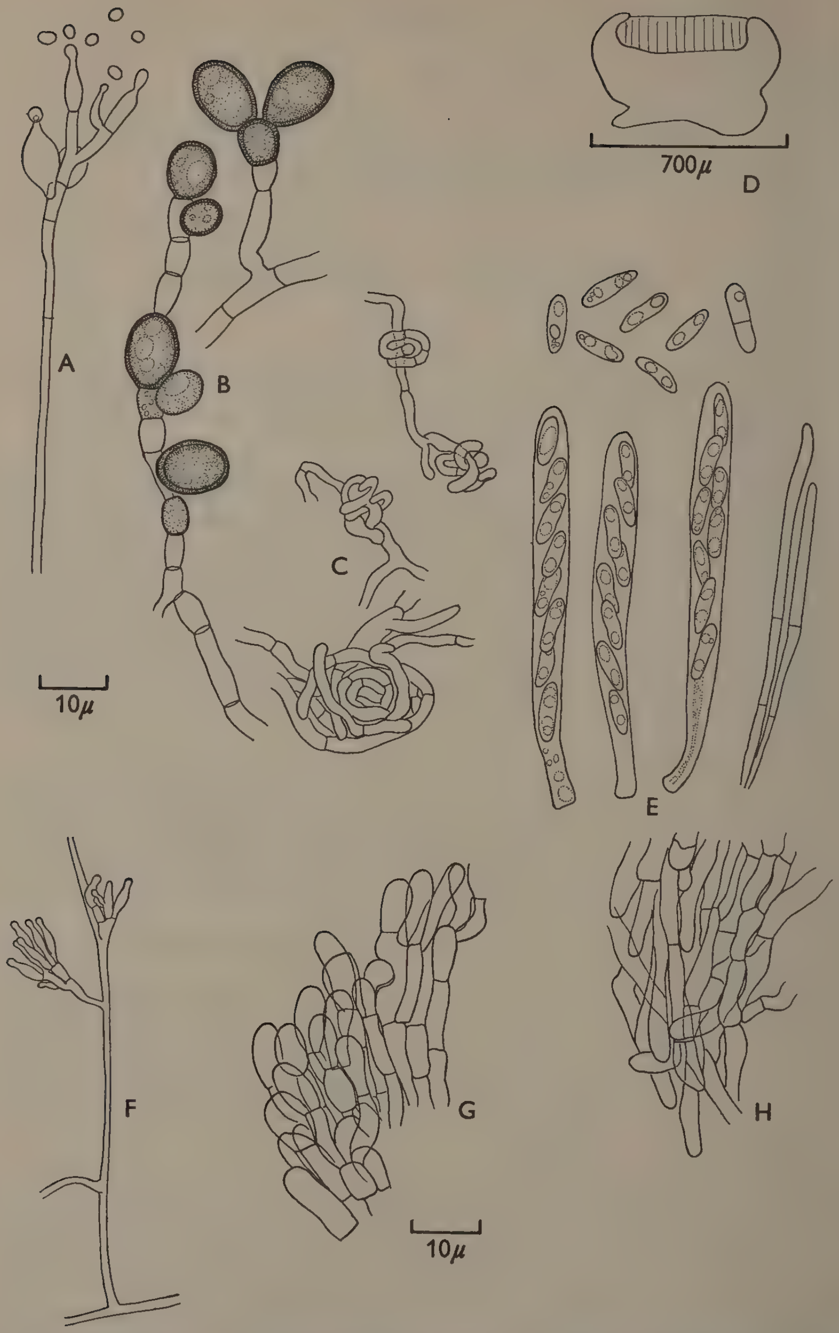

Fig. 1. *Anguillospora crassa* (Herb. Sheffield, no. 2520). A, Microconidiophores and microconidia formed in 9-month-old cultures derived from multi-spore inoculum of macroconidia; B, chlamydospores from the same culture; C, ascogonia from culture; D, section of apothecium formed in culture; E, asci, ascospores and paraphyses; F, microconidiophores from a 1-month-old culture derived from *Mollisia* ascospores, after 5 days' aeration in water; G, cells from the excipulum of the apothecium in section; H, surface view of excipulum. A, B, C, E to same scale. F, G, H, to same scale.

pressed air, sporulation was stimulated and on the fourth day macroconidia of *A. crassa* were found in both types of culture (Fig. 2A, B, C). The macroconidia develop as terminal swellings of the hyphae (Fig. 2B). The swollen tip becomes septate and curved in a sigmoid manner. Mature macroconidia from aerated cultures were 80–120 μ long (measured in a straight line across the span of the spore) and 9–11 μ wide at the widest part; there were up to twelve transverse septa. On germination a germ

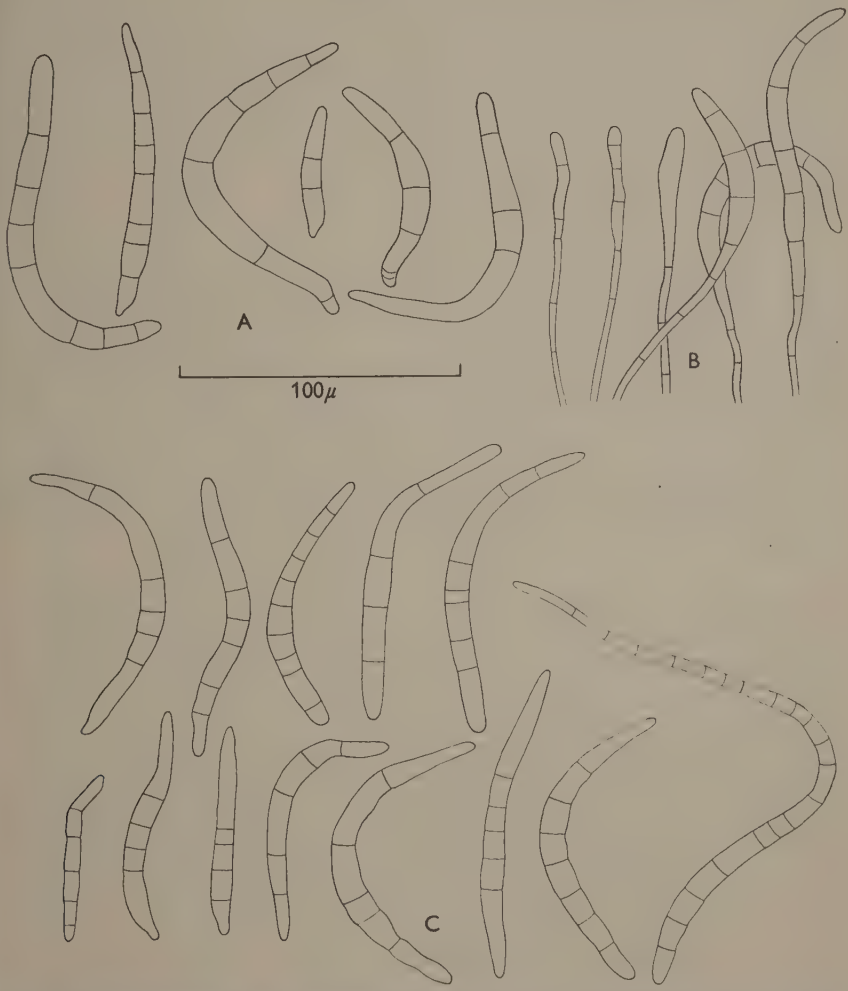

Fig. 2. *Anguillospora crassa* (Herb. Sheffield, no. 2520). A, Macroconidia formed in a 1-month-old culture derived from a single macroconidium of *A. crassa* after 5 days' aeration in water; B, development of macroconidia in a 1-month-old culture derived from *Mollisia* ascospores, after 4 days' aeration in water; C, mature macroconidia formed in 1-month-old cultures derived from *Mollisia* ascospores after 4 days' aeration in water. The spore on the extreme right has germinated.

tube is produced from one or both terminal cells. Ingold (1958) stated that 'naturally occurring spores from stream scum are hyaline, S- or L-shaped, 120–200 μ long and 15–20 μ wide in the middle region, tapering to 8–10 μ wide at the ends'. Spores produced in aerated cultures 'agreed fairly well with those seen in nature, but tended to be more variable in length and to have a reduced width'. The free spores figured by Ingold from his cultures measure 96–120 × 12–13 μ. Cultures prepared from *Mollisia* ascospores have been sent to Prof. Ingold who has kindly examined them and has confirmed that they agree with his concept of *A. crassa.*

Microconidia were also found after 5 days' aeration in both series of cultures (Fig. 1 F).

The evidence presented above demonstrates the genetical connexion between *A. crassa* and the *Mollisia* state.

Apothecia up to 1 mm. diam., at first cup-shaped, with a distinct inrolled margin, later convex; excipulum dark brown to grey, disk paler, white to cream. *Excipulum* composed of parallel pale brown hyphae about 5–8 μ wide, running out at the tips to oval or club-shaped round-tipped cells about 15–20 μ long and about 10–15 μ diam. *Asci* cylindrical to club-shaped, short-stalked, rounded at the tip, 8-spored, 56–68 × 5–6 μ. *Paraphyses* slightly exceeding the asci, narrowly clavate, tips rounded, septate, unbranched, 1·5–2 μ wide at the tip, at first hyaline, later pale brown. *Ascospores* irregularly biseriate, oblong-clavate or allantoid, usually with two prominent guttules, unicellular or rarely one-septate, smooth, hyaline, 7·5–10 × 2·5–3 μ.

Examination of the ascospore isolates has not provided conclusive evidence as to whether the fungus is homothallic or heterothallic. Apothecial rudiments have been found in some multi-ascospore isolates, but not in cultures derived from single ascospores.

Comparison of descriptions of *Mollisia* species known to grow on wood showed that a number of these are sufficiently similar to be considered as possible perfect states. Amongst those considered possible are *M. cinerea*, *M. benesueda*, *M. crumenuloides* and *M. lignicola.* Brefeld (1891) described the growth of *M. cinerea* in culture. Conidial formation began in 8 days: conidiophores bore groups of phialides at their tips, giving rise to ellipsoid hyaline microconidia 2·5–5 × 2 μ which do not match those formed in cultures of *A. crassa.* Le Gal & Mangenot (1956) described the conidia of various species of *Mollisia.* In old cultures of *M. benesueda* small black cushions 120–160 μ diam. developed. The stroma bore rigid filaments regularly divided at the apex into two phialides bearing long tubular collarettes. The phialides give rise to small globular phialospores 1·5–2 × 1·5–2·5 μ. Evidently this species does not match the cultures of *A. crassa.*

About twenty collections of *Mollisia* apothecia growing on rotten wood, especially from streams and stream margins, have been made. Cultures were prepared from ascospores and when about 1 month old the cultures were aerated vigorously in tap water to see whether they would produce *Anguillospora* conidia. So far no collection has been made which has proved to be the perfect state of *A. crassa.*

DISCUSSION

Webster (1959) has pointed out that although over forty species of aquatic hyphomycetes have been described, only two have been shown to have ascigerous states: *Flagellospora penicillioides* and *Heliscus lugdunensis*, both phialospore forms with *Nectria* perfect states (Ranzoni, 1956; Webster, 1959). The interest in the present observation is that an aquatic hyphomycete thallospore type should have a *Mollisia* ascigerous state. The polyphyletic nature of the aquatic hyphomycetes, already inferred from evidence of conidial development, is thus confirmed. Speculation about the occurrence in nature of these ascigerous states should await their discovery. Although a number of ascomycetes are known to grow submerged in water (Ingold, 1951, 1954, 1955), it is not know whether the perfect states of the above three species of aquatic hyphomycetes are aquatic or terrestrial. It is indeed an open question whether all the so-called aquatic hyphomycetes are exclusively aquatic, for there are various reports of their occurrence in terrestrial situations such as the surface of beech roots (Waid, 1954) and the 'leaf-carpet' of woods (Scourfield, 1940). In our laboratory we have encountered spores of *Varicosporium elodeae* and *Dendrospora erecta* on rabbit pellets, and have isolated the former as an aerial contaminant which fruited directly on agar. *Nectria lugdunensis*, which is known to occur on leaves in streams, was first described from pine bark (Webster, 1959). It thus seems likely that some of these hyphomycetes do live out of water and the possibility that their perfect states are not necessarily aquatic should certainly be considered when searching for them.

So far no example is known of a perfect state for an aquatic hyphomycete with a tetraradiate spore. I have, however, seen presumed ascogonia in cultures of the phialospore form *Lemmoniera aquatica*, and it seems possible that an ascigerous state for this fungus also exists.

I am most grateful to Prof. C. T. Ingold for his friendly advice and encouragement and for confirming the identity of the isolates. My thanks are also due to Mr W. D. Graddon for helpful comment, and to Mr W. G. Bramley for a number of *Mollisia* collections. I am indebted to the University of Sheffield Research Fund for financial assistance.

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STUDIES ON VESICULAR-ARBUSCULAR ENDOPHYTES

III. AN ENDOPHYTE OF *CONOCEPHALUM CONICUM* (L.) DUM IDENTIFIED WITH A STRAIN OF *PYTHIUM*

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(With 1 Text-figure)

A vesicular-arbuscular endophyte in the thallus of *Conocephalum conicum* (L.) Dum from a number of sites is described. The endophyte invades the thallus via the rhizoids and is then limited to the area adjoining the midrib.

A species of *Pythium*, resembling but not identical with *P. ultimum*, was consistently isolated from infected rhizoids and thalli by modifications of the section-embedding technique previously described. Isolation plates were frequently contaminated with other soil fungi unless special precautions were taken. The use of aureomycin and griseofulvin reduced the amount of contamination with bacteria and soil fungi, respectively.

Fungus-free thallus tips were prepared by allowing them to grow towards an overhead light source. These tips were removed, washed and planted in plugged boiling tubes containing a sterilized sand–soil mixture inoculated with a fungus culture. Of the fungi tested, only the *Pythium* isolate produced typical vesicles and arbuscules within the host thallus. All the other fungi were able to invade the rhizoids, and two (*Trichoderma viride* and an unidentified mycelium) invaded the lower cells of the thallus, but none of these reproduced the vesicular-arbuscular condition. It was concluded that the endophyte was identical with the species of *Pythium* isolated.

Among numerous green plants reported by various observers to contain endophytic fungi of the vesicular-arbuscular form are a number of liverworts (Butler, 1939; Kelley, 1950; Harley, 1959).

The first record of the presence of fungal hyphae in apparently healthy thalli of liverworts was that of Gottsche (1843) who observed coiled objects, which he did not at first recognize as hyphae, in cells of a species of *Preissia*. Leitgeb (1874) found that young sporocarps of *Ptilidium ciliare* were frequently infected with the mycelium of a fungus and Kny (1879) observed that, although the rhizoids of species of *Marchantia* and *Lunularia* were frequently traversed by fungal hyphae, these were present in the thalli only if the plants were growing in a soil rich in humus. Němec (1899), who recorded the presence of an endophyte in many leafy liverworts, also considered that incidence of infection depended upon the habitat.

The presence of endophytic fungi in *Conocephalum conicum* has been recorded by Czapek (1889), Beauverie (1902), Golenkin (1902), Cavers (1903, 1904), Denis (1919) and Nicolas (1925, 1927*a*). References to the presence of an endophyte in other thalloid liverworts (e.g. *Pellia*, *Marchantia* and *Lunularia*) are too numerous to list. The subject has been reviewed by Nicolas (1932).

Most of the voluminous literature on the fungal endophytes of liverworts is purely descriptive. Most authors describe the endophyte as being of the phycomycetous (vesicular-arbuscular) type and compare it with that found in the gametophyte of *Lycopodium* spp. and in the roots of many higher plants. Nicolas (1927*b*) considered the endophyte in *Marchantia polymorpha* to be a race of *Pythium debaryanum*. A few observers, however, describe septate hyphae in the thallus and particularly in the rhizoids. Some speculate as to the function and identity of the fungus concerned, but no one has hitherto succeeded in isolating a fungus which, when inoculated into the liverwort thallus under aseptic conditions, is able to re-establish the vesicular-arbuscular condition in the host.

MATERIAL

Conocephalum conicum was collected from a number of sites in limestone regions of Somerset and Gloucestershire and from the Malham Cove area in Yorkshire. Frequent collections were made from three sites: a limestone wall above a small stream at Lyncombe Vale, Bath; beneath a limestone bridge over a stream at Yanley, Long Ashton, Somerset; a bank bordering a ditch at Wotton-under-Edge, Glos. Cultivated material was also available in a cold glasshouse in the grounds of the University of Bristol.

DISTRIBUTION AND MORPHOLOGY OF THE ENDOPHYTE IN THE HOST

Hand sections of the liverwort were stained with aniline blue in lactic acid, or treated with a commercial preparation of pectolytic enzymes in order to free the hyphae from the host cells (Fraymouth & Hawker, 1952).

The incidence of the fungus in material collected from the natural habitat varied both from site to site and within the same patch of the liverwort, thus resembling the distribution in *Lunularia* rather than that in *Pellia*, all plants of which are reported to be infected (Ridler, 1922, 1923*a*, *b*). Plants from the cold glasshouse, where the liverwort had been maintained for many years by transplanting onto sterilized soil, were always free of infection.

Where the endophyte is present in the thallus, its distribution is always limited to a zone beneath the photosynthetic layer of the host and confined to the mid-rib area (Fig. 1). The actively growing margins of the thallus are invariably free from infection. The fungus gains entry to the thallus via the smooth-walled and the pegged rhizoids, and the hyphae traverse the parenchyma of the thallus to reach the midrib area. Frequently the fungal hyphae are present only in the rhizoids.

Longitudinal sections of the midrib show the presence of long, intercellular, aseptate hyphae, but the majority of the hyphae are intracellular and pass from cell to cell, being slightly constricted at the point of penetration of the host cell wall. Typical arbuscules and vesicles are formed, resembling those already described and figured for the endophytes of *Allium ursinum* roots and of certain ferns (Hawker, Harrison, Nicholls & Ham, 1957; Hepden, 1960).

A zone of red pigmentation of the cell walls of *Conocephalum conicum* frequently occurs along the midrib. This is not directly correlated with the presence of the fungus, since it may occur in thalli which are free of the endophyte and is occasionally absent in infected thalli. The colour was, however, most intense in heavily infected thalli. Uninfected cells in the 'fungal zone', and similarly situated cells in thalli lacking the endophyte, contained numerous starch grains but these were never seen in infected cells. Similar endophytes were seen in a number of other liverworts.

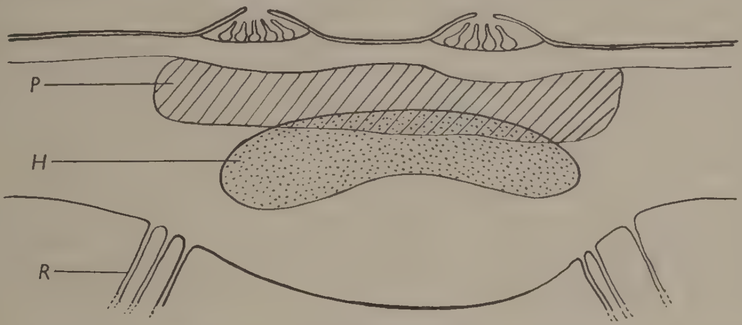

Fig. 1. T.S. thallus of *Conocephalum conicum* (diagrammatic). *H*, hyphal zone, containing hyphae of the endophyte; *P*, pigmented zone; *R*, rhizoids. Note position of hyphal zone at midrib of thallus.

ISOLATION OF A SPECIES OF *PYTHIUM* FROM INFECTED THALLI OF *CONOCEPHALUM CONICUM*

Attempts to isolate the endophyte of *C. conicum* by plating out rhizoids or pieces of the thallus, with the usual precautions to reduce contamination, gave a number of fungi, including common soil fungi or soil-inhabiting root parasites such as *Trichoderma viride* and species of *Verticillium*, *Fusarium* and *Rhizoctonia* and occasionally species of *Pythium*. Modifications of the section-embedding technique (Harrison, 1955) were finally employed for isolation of the endophyte. Aureomycin and griseofulvin were added to the isolation plates to reduce growth of bacteria and of vigorous soil fungi, respectively.

Isolation from rhizoids

Pieces of thallus were washed under the tap and brushed to remove soil particles. They were then shaken in several changes of sterile water and transferred to a drop of sterile water (containing a little 'Stergene' to facilitate separation of the rhizoids) in a cavity slide, and examined under the microscope. Infected rhizoids were transferred by means of a home-made micromanipulator, of the type described by Goldacre (1954), to a slide coated with a thin layer of water agar or of 3 % malt agar plus aureomycin and/or griseofulvin or to a hanging drop culture. The cultures were examined daily and hyphae growing out of the rhizoids were picked up and transferred to plates of 3 % malt agar.

Isolation from thallus

The material was treated with a weak hypochlorite solution and rinsed in sterile water, or was washed as already described, and sections were cut under aseptic conditions. These were embedded in a drop of water agar plus aureomycin on a coverslip, which was then inverted and sealed to a glass ring, as in the preparation of a hanging drop culture. When hyphae grew out of the infected cells, the tips were picked off and transferred to plates of 3 % malt agar plus aureomycin and griseofulvin.

From both rhizoids and thallus, various soil fungi including a species of *Pythium* were isolated. *Pythium* was obtained only when treatment with hypochlorite was omitted. It has already been pointed out (Harrison, 1955) that vesicular-arbuscular strains of *Pythium* from *Allium* roots are particularly sensitive to this substance.

All the isolates of *Pythium* were similar. They closely resembled that isolated from roots of *Allium ursinum* and some other plants (Hawker *et al.* 1957). These authors considered their isolates to be a strain of *Pythium ultimum* Trow but a further examination of the isolate was kindly made by Miss G. M. Waterhouse, who considered it to have some resemblance to *P. ultimum* but not to be identical with it.

INOCULATION OF FUNGUS-FREE THALLUS TIPS OF *CONOCEPHALUM CONICUM* WITH *PYTHIUM* AND OTHER ISOLATES

For inoculation experiments, fungus-free material of the host plant was necessary. It has already been stated that the material from the University glasshouse contained no endophytic fungus. It was, however, difficult to obtain thallus tips free from soil contamination. Moreover, it was desirable that material for inoculation should be taken from plants known to be capable of becoming infected naturally and that it should be uniform. If fresh thalli were placed in a deep dish and illuminated from above, the thallus tips turned upwards away from the older parts of the plant. Such young tips, as already stated, contained no endophytic fungus. Suitable thallus tips were cut off, thoroughly washed in several changes of sterile water and planted out on a sterile sand and soil mixture in plugged boiling tubes. Varying amounts of 1-week-old sand and oatmeal culture of the isolate to be tested were added to the soil–sand mixture before the liverwort thallus tips were planted.

Thallus tips were inoculated in this way with isolates of *Pythium*, *Trichoderma*, *Verticillium*, *Penicillium*, *Fusarium* and *Rhizoctonia*, and also with an unidentified mycelium obtained from infected rhizoids and thalli of *Conocephalum conicum*. Comparable controls were left uninoculated.

The results of this experiment are shown in Table 1. After 7 weeks from planting, only the thalli inoculated with *Pythium* contained a typical vesicular-arbuscular endophyte. Intercellular and intracellular hyphae, vesicles and arbuscules were present and resembled those seen in naturally infected plants. Six weeks later, the arbuscules still showed no sign of disintegration and the liverworts were growing satisfactorily. Observations soon after inoculation confirmed the observation, made with

Table 1. *Results of inoculating fungus-free thallus tips of* Conocephalum conicum *with* Pythium *and with other fungal isolates from naturally infected thalli*

Fungal inoculum	Days after inoculation	No. plants examined	Condition of thallus	Fungus surrounding rhizoids	Rhizoids penetrated by hyphae	Fungus in thallus
None	44	2	Healthy	None	None	None
None	91	4	Healthy			
None	98	3	Healthy			
Pythium sp.	44	5	Healthy	+	+	Branched, aseptate hyphae, few vesicles, no arbuscules
Pythium sp.	49	5	Healthy	+	+	Branched, aseptate hyphae, many vesicles, few arbuscules
Pythium sp.	91	3	Healthy	+	+	Branched, aseptate hyphae, many vesicles, many arbuscules
Unidentified septate mycelium	49	3	Healthy	+	+	Coiled, septate hyphae, no vesicles or arbuscules
Unidentified septate mycelium	63	2	Healthy	+	+	Coiled, septate hyphae, no vesicles or arbuscules
Unidentified septate mycelium	91	3	Healthy	+	+	Coiled, septate hyphae, no vesicles or arbuscules
Verticillium sp.	49	3	Healthy	++	−	None
Verticillium sp.	91	3	Healthy	++	+	
Botrytis cinerea	20	4	Healthy	++	−	
Botrytis cinerea	63	3	Healthy	++	+	
Fusarium sp.	20	3	Healthy	+	−	
Fusarium sp.	63	4	Healthy	+	+	
Penicillium sp.	49	2	Healthy	++	−	
Penicillium sp.	91	3	Healthy	++	−	
Trichoderma viride	22	2	Yellow, unhealthy	+	+	Hyphae in ventral cells
Trichoderma viride	49	3	Tissue rotting	+++	+	Ramifying septate mycelium

− = no hyphae. + = few hyphae. ++ = many hyphae. +++ = dense mass of hyphae.

naturally infected material, that the endophytic fungus enters via the rhizoids.

No other fungus among those tried induced the appearance of a typical endophyte in the host plant, and none other formed either vesicles or arbuscules. All of them, however, enveloped the rhizoids, and some penetrated into them. The unidentified septate mycelium penetrated the ventral cells of the thalli and produced loose coils in some of them, but the condition in no way resembled that of the naturally infected thalli. *Trichoderma* also penetrated the ventral part of the thalli and caused yellowing and rotting of the tissues. The control plants remained healthy and uninfected.

It is clear that only *Pythium* sp., amongst the various fungi isolated and tested, could form a vesicular-arbuscular mycotrophic association with the thalli of *Conocephalum conicum*. A criticism of the experiment just described is that the cut edge of the thallus was not allowed to heal before inoculation, and that this may have allowed some fungi to enter which would not normally do so. The *Pythium*, however, was seen to enter via the rhizoids rather than via the cut edge of the thallus.

Discussion

The descriptions of the endophyte given by different observers vary for the same or for different species of liverwort. From the present study, it is clear that a number of fungi are able to invade the rhizoids and that some of these are able to penetrate the lower part of the thallus, with varying effect. Among the fungi tested, only a strain of *Pythium* reproduced the appearance of the vesicular-arbuscular endophyte seen in naturally infected thalli. It is thus clear that this fungus is one of those causing this relationship, but it is not impossible that under different conditions, or with different species of liverwort, other fungi may behave similarly.

The relation between fungi and liverworts as described by numerous investigators varies from destructive parasitism to the vesicular-arbuscular mycotrophic condition, which does not visibly harm the plant; chance invasions of the rhizoids are also common. The vesicular-arbuscular condition is widespread. Some observers (e.g. Chaudhuri, 1925) claim that it actually benefits the liverwort. It cannot, however, be essential for growth under all conditions, since in many habitats liverworts grow well in the absence of any endophyte. Other observers (Ridler, 1922, 1923*a*, *b*) consider the endophyte to be harmful. It may well be that the same fungus is beneficial or harmful according to the conditions under which the host is growing. The present study provides no clear evidence of either harmful or beneficial effects of the only fungus (*Pythium* sp.) which, when inoculated into thallus tips, induced the formation of the typical vesicular-arbuscular endophyte. The intensification of the red pigmentation of the cell walls of the pigmented zone of the liverwort in the presence of the endophyte, and the absence of starch from invaded cells, suggests both a chemical reaction to the invading hyphae and a depletion of host food reserves by the fungus. The limited distribution of the latter within the thallus further suggests some mechanism of resistance to further spread. No significant inhibitory effect of host thallus or thallus extracts on the growth of the *Pythium* isolate could be detected.

It has been suggested by Hawker *et al.* (1957) and by Hepden (1960) that the *Pythium* isolates from *Allium ursinum* roots and from ferns are able to enter the appropriate host and form a vesicular-arbuscular relationship only when well nourished before reaching the host plant. In the present study, attempts (not given in full here, but described by Harrison, 1957) to inoculate thallus tips on damp filter paper, by means of small pieces of agar culture of *Pythium*, were unsuccessful. The fungus invaded the rhizoids but was unable to penetrate further. This suggests that the liverwort endophyte, too, is only able to become mycotrophic under conditions of good nutrition, and agrees with the observations made by several previous authors (e.g. Kny, 1879) that the endophyte occurs in some species of liverwort only when these are growing in soil rich in humus.

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PHOMA SPP. ASSOCIATED WITH PAINTED SURFACES

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A survey of *Phoma* spp. attacking painted surfaces was carried out: *Phoma saprophytica* sp.nov. is described and *Aposphaeria violacea* Bertel (syn. *Phoma pigmentivora* Massee) is transferred to *Phoma*. Though both species are common in glasshouses, no evidence of pathogenicity to a range of common plants was shown by either of them.

Moulds have been known for many years to cause disfiguration of painted surfaces, the principal form encountered being a general darkening and also spotting of the paint film. This discoloration is directly due to the dark nature of the mycelium of the predominant fungi, *Pullularia pullulans* (de Bary) Berkh. and *Cladosporium herbarum* Link, though disfigurement may also be caused by pigment diffusing from the mycelium into the paint vehicle. This type of discoloration was first reported by Bertel (1904), who identified *Aposphaeria violacea* Bertel as the cause, red violet blotches being produced on interior glasshouse paintwork. The fungus was redescribed as *Phoma pigmentivora* Massee (1911). Spasmodic reports of it have since been made (Grove, 1913; Haensler, 1921; Nicot, 1951, and Klens & Lang, 1956), but little work has been done on its physiology in relation to attack of paint films. This paper presents a taxonomic treatment of *Phoma* spp. isolated during an investigation of the mode of fungal attack of paint films.

Phoma spp. 'attacking' paint films are known to have a wide geographic distribution: Czechoslovakia (Bertel, 1904), U.S.A. (Haensler, 1921), Germany (Wurth, 1940), and France (Nicot, 1951). Isolates have been obtained by the author at sites across Britain (Table 1) and more recently in Nova Scotia, Canada.

MATERIALS AND METHODS

Isolates were collected in general from indoor situations in which both high temperature and humidity prevailed. Cultures nos. 18, 19, 20, 49, 50, 51, 52*a*, 53, 54, 55, 56, 59 and 60 were all isolated from glasshouses, nos. 3, 14, 15 and 61 from washrooms and no. 62 from a hospital sterilizing room (Table 1). A single isolate (no. 52) was obtained from external paintwork where the fungus completely covered the wall of a double storey building.

Disfigured paint chippings were shaken with sterile water and this inoculum used to prepare dilution series for cornmeal and malt (Oxoid) agar plates. Cultures were made on cornmeal agar slopes by mass transfer of mycelium and pycnospores and maintained at 25° C. in darkness unless otherwise stated.

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Table 1. *Sources and comparative sizes (in μ) of isolates*

				Spores (mean values per 50 spores from malt agar cultures except where indicated)		Pycnidia (mean value of 50 measurements per strain)	
	Source	Substrate	Locality	Length Range (mean)	Breadth Range (mean)	On malt agar Range (mean)	On paint Range (mean)
Phoma violacea							
	Bertel (1904)	White paint	Prague	(6·8)	(3·2)	(260)	—
	Massee (1911) P	White paint	R. B. G., Kew	4–6 (P)	2–2·5	—	P 125–150
	Haensler (1921) P	White paint	New Jersey, U.S.A.	4–6 (P)	2·1–2·5	160–320	P 50–150
	Nicot-Toulouse (1953) P	Oil paint	Normandy	4·6–5 (P)	2–2·5	—	—
1	CMI (I.M.I. 49948)	White paint	—	3·3–6·1 (4·7)	1–3·1 (2)	90–350 (170)	P 90–160
2	PRS	White lead paint	—	Mycelium only	—	—	—
3	LSHT BB 257 (G. Smith)	Paint	H.M.S. Vanguard	4·1–6·6 (5·4)	1·7–2·8 (2·2)	50–120 (80)	T 60–180 (100)
4	Birmingham University F154/1	Human mouth	—	4–6 (4·8)	1·5–3·3 (2·2)	75–240 (180)	T 60–195 (115)
6	PRS	Paint	—	3·9–6·3 (4·8)	1–2 (1·5)	60–150 (90)	—
7	CBS (K. Cartwright)	Paint	—	3·6–6·8 (5·3)	1–2·5 (1·8)	90–270 (180)	T 45–335 (110)
8	MM 1508 (J. Nicot)	Cement	Paris	Mycelium only	—	—	—
9	MM 74 (J. Nicot)	Oil paint	Normandy	Mycelium only	—	—	—
13	PRS	Paint	—	3·7–5·5 (4·5)	1·4–2·6 (1·9)	90–315 (200)	P 60–120
14	D.E.E.	Paint	Buckfastleigh, Devon	4·1–6·5 (5·3)	1·9–3·6 (2·5)	45–120 (85)	—
15	D.E.E.	Paint	St Ives, Cornwall	3·8–6·8 (5·1)	1·7–2·6 (2·2)	60–180 (130)	—
18	D.E.E.	White lead paint	Bishopsteignton, Devon	4–6·4 (5·0)	1·6–3·3 (2·3)	90–330 (180)	P 75–135
19	D.E.E.	White lead paint	Kingsteignton, Devon	4–5·8 (4·9)	1·5–2·4 (2)	60–225 (115)	P 60–120
20	D.E.E.	White lead paint	Newton Abbot, Devon	3·8–6·1 (4·9)	1·5–2·4 (2)	75–210 (100)	P 35–85
37	CBS (K. Cartwright)	Paint	—	Mycelium only	—	—	—
49	D.E.E.	Paint	R. B. G., Kew	3·1–5·4 (4·1)	1·2–2·1 (1·6)	50–225 (130)	—
50	D.E.E.	Paint	R. B. G., Kew	3·5–6·7 (5·5)	1·5–3 (2·4)	105–210 (150)	—
51	D.E.E.	White lead paint	Exeter, Devon	3·4–6·1 (4·5)	1·5–2·5 (2)	60–150 (100)	P 35–90
52	D.E.E.	White paint	Tavistock, Devon	3·3–6·3 (5·3)	1·3–2·6 (1·9)	110–440 (250)	T 90–300
52*a*	D.E.E.	White lead paint	Tavistock	Mycelium only	—	—	—
53	D.E.E.	White lead paint	Rockbeare, Devon	4·1–6·7 (5·5)	1·7–2·6 (2·1)	165–300 (230)	P 45–150
54	D.E.E.	White lead paint	R. B. G., Edinburgh	3·2–5 (3·9)	1·4–2·4 (1·9)	90–255 (170)	P 30–60

Table 1 (*cont.*)

	Source	Substrate	Locality	Spores (mean values per 50 spores from malt agar cultures except where indicated)		Pycnidia (mean value of 50 measurements per strain)	
				Length Range (mean)	Breadth Range (mean)	On malt agar Range (mean)	On paint Range (mean)
Phoma violacea (cont.)							
55	D.E.E.	White lead paint	Bot. Gdns, Birmingham	4–7·1 (5·6)	1·7–3·1 (2·3)	150–300 (200)	P 40–60
58	D.E.E.	Paint	Exmouth, Devon	3·7–4·4 (4·1)	1·8–2·1 (2)	75–120 (100)	—
59	D.E.E.	White lead paint	Topsham, Devon	4·1–6·6 (5·5)	1·5–2·4 (2)	195–480 (370)	P 30–45
60	D.E.E.	Paint	Botany Dept., Univ. Birmingham	3·6–6·5 (5)	1·3–2·5 (2)	100–450 (150)	P 40–135
61	D.E.E.	Emulsion paint	Univ. Coll., London	3·8–6·1 (4·9)	1·6–2·6 (2·2)	75–225 (150)	P 20–35
62	D. Snow	Paint	Ascot, Berks	3·5–5·7 (4·7)	2·1–3·5 (2·7)	120–300 (220)	—
Phoma saprophytica							
5	D.E.E.	White lead paint	Air contaminant, Exeter	4·4–9·4 (5·9)	2·3–3·9 (3·1)	60–300 (135)	—
16	Rec. I.C.I. Ltd., Bracknell, Berks	Cement	—	4·7–8·2 (6·2)	2·4–4·1 (3·2)	60–190 (120)	—
57*a*	D.E.E.	Paint	Brewery, Exeter	5–7·7 (6·2)	1·9–3·6 (2·9)	60–210 (110)	—
60*a*	D.E.E.	Paint	Greenhouse, Univ. Birmingham	3·8–9·9 (6·1)	2·2–3·9 (3·1)	60–135 (90)	—
63	D.E.E.	Soil	Exeter	4·5–8 (6)	2·2–3·6 (3·0)	50–120 (90)	—
Phoma spp.							
53*a*	D.E.E.	White lead paint	Rockbeare, Devon	3·7–6·3 (5)	1·6–2·5 (2·1)	50–150 (90)	—
56	D.E.E.	Paint	Univ. Glasgow	3·9–6·6 (5)	1·7–2·5 (2·1)	90–300 (190)	P 45–165
Diplodina sp.							
57	D.E.E.	Paint	Brewery, Exeter	3·8–6·1 (4·9)	1·4–2·5 (2·1)	75–150 (110)	P 40–165

Abbreviations: CBS, Centraalbureau voor Schimmelcultures. CMI, Commonwealth Mycological Institute, Kew. LSHT, London School of Hygiene and Tropical Medicine, London. MM, Museum National d'Histoire Naturelle, Paris. PRS, Paint Research Station, Teddington. D.E.E., D. E. Eveleigh. CM, Cornmeal agar. P, Original paint. T, Test white lead paint.

N.B. Of three sets of blue-stained paint chippings, received from the Paint Research Station, which could not be cultured, no pycnidia were present on two sets while the other had pycnidia 55–120 μ.

The status of the *Phoma* isolates was studied by pathological, morphological and physiological criteria, with due regard to the wide variability characteristic of the genus. Comparative studies, including laboratory tests on the ability of the cultures to attack paintwork, were carried out (Eveleigh, 1961) with the fungi listed (Table 3).

Of the thirty-six isolates examined, twenty-eight were identified as *Aposphaeria violacea*, seven as *Phoma* spp. (five were believed to represent a new species), and one as *Diplodina* sp. (Table 1); the last had erumpent pycnidia and uniseptate spores. The spores of all other isolates were non-septate with average lengths within the range $3{\cdot}9\,\mu$ (no. 54)–$6{\cdot}2\,\mu$(no. 16). Origin of pycnidia and conidiophore morphology were found to be characters of no taxonomic value, since pycnidial origin in all strains was meristogenous (Kempton, 1919), while conidiophores were small and not distinctive. Physiological characters, such as the utilization of carbohydrates and nitrogenous substrates did not yield any satisfactory distinguishing features (Eveleigh, 1961). Liquefaction of gelatin has been used to segregate *Phoma* strains (Dennis, 1946; Grimes, O'Connor & Cummins, 1932), but this test gave no distinction among the pycnidiaceous strains under investigation, liquefaction being less than 5 mm. depth after 4 weeks' growth in all instances. Two of the strains, nos. 37 and 59, liquefied the gelatin to a depth of 5–20 mm. in 4 weeks, a difference being noted between mycelial (no. 37) and pycnidial (no. 7) strains of the same isolate.

Inoculations were made on a number of plants known to be susceptible to certain Phomae. In the first group of experiments healthy and artificially injured (rubbed with carborundum) young plants were sprayed with a spore or mycelial suspension and then grown in polythene 'humidity' chambers for 3 weeks. The plants used were tomato (cultivar Stonor's Moneymaker), lettuce (Continuity Cabbage), cucumber (Improved Telegraph), cabbage (Rainham), onion (Ailsa Craig), *Senecio vulgaris*, *Digitalis mertonensis*, *Armeria formosa*, *Sorghum vulgare* and *Bellis perennis*. Five replicates of each species were used in each test, all *Phoma* isolates being tested. No infections were recorded. In the second group of experiments a mycelial block was placed aseptically under a 'skin flap' of surface sterilized apple fruits (isolates 1, 2 and 3) and Doon Star potato tubers (all isolates) known to be susceptible to 'gangrene' associated with *P. solanicola* rot (Dennis, 1946). The wounds were covered with sterile cotton wool and the tubers stored at room temperature for 6 weeks. No infections occurred but a control inoculation with *P. solanicola* produced a small hard rot in the potato tubers. Therefore the isolates appeared to be only saprophytic.

NOMENCLATURE AND TAXONOMY

Phoma and *Aposphaeria*, as interpreted today, do not conform to their original descriptions (*Aposphaeria* Berk., in *Outlines of British fungology*, p. 315, 1860; *Phoma* Fr. in Fries, *Systema mycologicum* II, ii, 546, 1823). *Phoma* has been conserved *sensu* Saccardo (*Michelia*, **2**, 4, 1880) with *P. herbarum* Westend. ex Sacc. (*Syllog. fung.* **3**, 133, 1884) as the type species (*International code of botanical nomenclature*, 1956). *Aposphaeria* was also proposed for conservation (*International rules of botanical nomenclature*, 1935, p. 39) *sensu* Saccardo (*Michelia*, **2**, 4, 1880). A British Mycological Society Nomenclatorial Committee (These *Trans.* **23**, 287, 1939) supported this recommendation, proposing as the type species *A. pulviuscula* (Sacc.) Sacc. However, in the *International rules* for 1935 the type species proposed was *A. pinea* Sacc. (*Michelia*, **1**, 126, 1877) but this was never finally approved. The criterion used to separate the two genera is that pycnidia of *Aposphaeria* are characterized by being superficial from inception and never erumpent, while those of *Phoma* generally develop subepidermally and later become erumpent.

A. violacea and *P. pigmentivora* were placed in synonymy by Grove (1935, pp. 140–141); Nicot-Toulouse (1953) agreed. The *Phoma* isolates from paint obtained in the present investigation all had plainly erumpent pycnidia, which were increasingly so as the attack became more severe. If the erumpent character is accepted as a sound taxonomic criterion (Saccardo, *Syll. fung.* 1822–1931, lists 153 species of *Aposphaeria* with this character), *A. violacea* cannot be left in *Aposphaeria*, but must be placed in *Phoma*. (Bertel (1904) used *Aposphaeria* sensu Sacc., *Michelia*, **2**, 4, 1880.)

On this consideration *Aposphaeria violacea* Bertel is transferred as

Phoma violacea (Bertel) comb.nov.

Basonym *Aposphaeria violacea* Bertel, *Öst. bot. Z.* **54**, 205, 233, 288, 1904.
Synonym *Phoma pigmentivora* Massee, *Kew Bull.* **8**, 325, 1911.

On malt agar growth variable according to the strain; slight aerial mycelium and abundant pycnidia or a high aerial mycelium and few pycnidia—with intermediates. Segregation of pure mycelial lines occurs. Coloration on malt agar pink-red to dark brown. Always some degree of red pigmentation on cornmeal agar, the pigment being soluble in oil, producing a characteristic red stain. Probably saprophytic only. *Pycnidia* are formed meristogenously and generally have one, but occasionally 2–3, ostioles. On paint pycnidia become increasingly erumpent with further growth; brown-black, subglobose, parenchymatous in texture, 30–400 μ according to the strain and the substrate. *Conidiophores* small and inconspicuous. *Spores* normally orange-pink in mass, liberated at maturity, unicellular, hyaline, ellipsoid, often biguttulate, $3{\cdot}1$–$6{\cdot}8 \times 1$–$3{\cdot}6$ (av. $4{\cdot}9 \times 2{\cdot}1$) μ.

Habitat. Red-violet colonies often several cm. diam. on white oil paints, frequent in glasshouses. The stain can also form large diffuse patches on exterior paintwork.

Lectotype: Massee specimen, Herb. Kew. Material has been deposited at the Commonwealth Mycological Institute, Kew (Herb. I.M.I. 85466–69), Exeter University, and Botany and Plant Pathology Laboratory, Science Service, Ottawa.

The species classification of *Phoma* generally adopted is that of Saccardo (1884–1931), parasitic species being based on host specificity. This classification is acknowledged to be inconsistent since individual 'species' are pathogenic to a range of host plants (Wollenweber & Hochapfel, 1936). Saprophytic Phomae have received specific epithets according to the substrate attacked, e.g. *P. chartarum* Berk. & Curt.

Variation shown by a single species may be great, as described for *P. lingam* (Tode) Desm. (Pound, 1947) and *P. betae* Frank (Tompkins & Pack, 1932), variations being manifest in morphology, size of spores and pycnidia, colony topography, pigment production and pathogenicity. Such variation may arise from heterokaryosis, as *Phoma* spp. exhibit the 'dual phenomenon' (Hansen, 1938), and has been induced by exposure to nitrogen mustard gas (Calvert *et al.* 1949; Gasiorkiewicz, Larson, Walker & Stahmann, 1952). The complexity of the genus has led to critical taxonomic investigations by Grimes *et al.* (1932), Dennis (1946), and Malcolmson (1958).

Variation in Phoma violacea

Isolates were subdivided into three groups according to spore colour in mass, a character which can be variable within a species (Dennis, 1946).

GROUP 1. Spores orange-pink, isolates 1, 3, 4, 6, 7, 14, 15, 18–20, 50, 51, 52, 53, 55, 58, 59, 60, 61, 62; mycelial isolates 2, 8, 9 and 37.

GROUP 2. Spores yellow, isolate 13.

GROUP 3. Spores milky-white, isolate 54.

Mycelial isolates, 8, 9 and 37, were considered as a section of group 1, having arisen from pycnidiaceous cultures with orange-pink spores in mass.

GROUP 1. Morphological variants were evident in this group, differences in topography and pycnidial production being observed on malt agar. No distinction could be made with regard to spore lengths (no. 25, av. 3·8 μ to no. 59, av. 5·5 μ) which did not vary appreciably with different media, nor regarding colony topography on Czapek-Dox or cornmeal agars. Growth on cornmeal agar was never aerial, the hyphae growing deeply into the agar and, though hyaline, producing various intensities of the orange-pink pigment. Production of pycnidia was stimulated by growth on cornmeal agar, except in the mycelial strains, pycnidial size varying between isolates, e.g. no. 20, 45–75 μ and no. 53, 105–180 μ (Table 1). Growth on Czapek-Dox agar was a compact aerial turf. The peripheral zone of the colony on Dox agar tended to be pink, but the older zones became greenish black, especially those hyphae deep in the agar. Pycnidia formed superficially but were virtually covered by mycelium. Mycelial isolates produced orange-red pigment, especially when grown on Czapek-Dox agar, but also to varying degrees on all other media tested, e.g. malt, potato dextrose and rice agars and on mineral-salts agars incorporating single carbohydrates (including galactose, maltose, lactose and mannitol).

Four sections (A–D) were distinguished on colony topography on malt agar, which remained constant for the individual isolates during the investigation.

Section A. Isolates 1, 6, 18, 49, 50, 58, 59 and 60

Aerial mycelium was initially high, tufted and white, the young colony edge always having this type of growth. The centre became black after several days (4 days with 18) and collapsed to reveal the brown-black pycnidia on the agar surface; 58 turned black almost immediately. Isolates did not produce pycnidia abundantly on malt agar. The peripheral edge of the colony sometimes had a pink tinge, but the main mycelium in the agar was black. The isolates in this section appeared to be similar to Bertel's strain.

Section B. Isolates 7, 14, 15 and 19

On malt agar they produced a flat brown mycelium. The abundant, small, brown-black pycnidia, partially immersed in the agar, were produced after 4 days, and when 8 days old were sporing freely. Aerial myclium was virtually non-existent. The growth rate was faster than that of section A isolates, and colonies would eventually grow to the edge of the Petri dishes (90 mm.) without staling. There was production of much brown pigment by isolates 7 and 19 which diffused from the mycelium and crystallized in the agar. In all isolates the brown pigment could be seen dispersed in the hyphae and crystallized on the walls of the dead hyphae.

Section C. Isolates 3, 4, 20, 51, 52, 61 and 62

Colony topography was intermediate between that of A and B. All isolates in this section produced abundant pycnidia, the size of which varied considerably between strains (Table 1). The pycnidia were at first covered by a medium aerial mycelium, which later collapsed to reveal large numbers on the agar surface. In general, pycnidia were black, though those of isolates 3 and 4 were always brown. Aerial mycelium was white, later black, turning quickly in some isolates (52), while the hyphae in the agar were either brown or black-brown. On ageing the whole colony turned black.

Section D. Isolates 2, 8, 9, 37 and 55

These were all mycelial and all were characterized by abundant production of red pigment on malt, Czapek-Dox and especially starch-based media, the pigment diffusing into the medium. Colony growth was a rather dense aerial turf. Growth rates varied considerably: thus mean colony diam. of isolate 2 on malt agar in 12 days was 38 mm. in contrast to 69 mm. for 9. Cultures, 8, 9 and 37 were received in the mycelial state though originally known to have been pycnidiaceous. They may have been obtained by selective culturing from mycelial sectors (see later). Isolate 2 was from purple-stained white lead paint; no pycnidia were found on the paint and all isolates obtained were mycelial. Isolate 55, included in this subsection though it did in fact produce a few pycnidia on cornmeal agar, developed a slight aerial mycelium on malt agar, the greater part of it forming deeply in the agar and being highly pigmented.

GROUP 2. Colony on malt agar a high tufted pinkish white mycelium which began to turn black after 4 days' growth. Growth was relatively slow (35 mm. in 12 days at 25° C.). A few pycnidia were formed on the surface and spores exuded after 12 days growth as yellow tendrils. On Czapek-Dox agar a dense aerial mycelium formed with a more pronounced pink tinge than that on malt agar. The reverse was pink at first, finally black. On cornmeal agar the colony was hyaline and developed deep in the agar; hyphae were at first a delicate pink, but finally became greenish black. No pycnidia were present on the infected paint sample received.

GROUP 3. On malt agar a fluffy aerial mycelium was produced, at first white and in 4 days becoming greenish black. Black pycnidia matured after 4 days and exuded spores in a milky white slime. The aerial mycelium finally collapsed, and when 12 days old the colony appeared black, with numerous discrete, sporing, superficial black pycnidia. Growth rate was about average for the *Phoma* isolates examined in this investigation, colony diam. 72 mm. after 12 days at 25° C. The pigment, confined generally to the mycelium, dissolved in oils producing a red coloration. On Czapek-Dox agar the aerial mycelium became thick and did not collapse. Cultures on cornmeal agar produced only hyphae deep in the agar, which appeared hyaline, with an orange tinge due to pigment production. Brownish black pycnidia were formed at the agar surface.

The nuclei

The possible occurrence of the 'dual phenomenon' (Hansen, 1938) and heterokaryosis in the *Phoma* isolates was suggested by the variable colonial form, with regular appearance of mycelial sectors of stable mycelial strains (section D). The production of pycnidia in certain sectors and sometimes in a complete colony was sometimes confined to a few main hyphae, the pycnidia thus appearing in lines radiating from the colony centre; this was especially so in isolates 7, 19, 20 and 62. This may have been due to the hyphae possessing only nuclei of the 'C' type (Hansen, 1938), i.e. pycnidial inducing nuclei. Nuclei were stained in basic fuchsin (DeLamater, 1948) and azur A (Flax & Pollister, 1949), the former method proving superior. Spore smears, germinated spores on thin 'malt' smears, and wax-embedded colony sections were stained. Pycnospores stained well, though the mycelial nuclei stained only occasionally.

Hansen (1938) demonstrated that *Pyrenochaeta terrestris* had binucleate pycnospores, giving rise to the 'dual phenomenon'. Gasiorkiewicz *et al.* (1952) have since described a strain of this fungus with only uninucleate pycnospores. Hansen demonstrated 'dual phenomenon' in all the 104 strains tested of *P. terrestris*, eight out of twelve isolates of *Phoma* spp. and four out of seven isolates of *Phyllosticta* spp. He showed that *Verticillium alboatrum* produced mainly uninucleate conidia, but occasionally binucleate ones were present, to which he attributed the occurrence of the 'dual phenomenon' which he had demonstrated for this species. The situation in the *Phoma* isolates in the present study was similar to this, in that uninucleate pycnospores predominated in all strains tested, but appreciable numbers of binucleate spores also occurred. Binucleate conidiophores were never actually demonstrated. Pycnospore nuclei apparently having four chromosomes per nucleus were demonstrated in isolates 52 and 61. The mycelium was composed mainly of uninucleate cells, but there were heterokaryons in fusions of isolates 3, 51 and 62. Fusions between the hyphae of mycelial isolates 2 and 9 were seen in cultures growing in the same Petri dish, but nuclei were never demonstrated in these instances by the staining technique given above. All isolates showed hyphal fusion soon after pycnospore germination. These cytological studies indicate that the observed variation in colony form may be attributable to the mechanisms involved in both the 'dual phenomenon' and heterokaryosis.

The pigment

Pigment formation of all strains of *Phoma violacea* was dependent on light and on certain types of media for its production. On white lead paint the colours ranged from red to violet (Table 2). Massee (1911) stated that in culture the mycelium remained colourless on 'ordinary nutritive media' and also that white lead was 'the constituent from which the fungus produces a by-product, the purple colouring matter'. Haensler (1921) obtained rosy pink cultures on 'culture media'. More detailed reports show that the pigment is produced on meat gelatin agar (Bertel, 1904) and on starch-based media, especially oatmeal agar (Nicot-Toulouse, 1953). Results of the present investigation support Nicot-Toulouse's observation, especially with regard to the use of cornmeal agar. Variation both in the amount and colour of the pigment occurred between isolates. In general, slight pigmentation developed at the periphery of the colony on Czapek-Dox agar, except with mycelial isolates 2, 8, 9 and 37, in which strong pigmentation developed over the whole colony. Pigment production appears to be linked in some way with this mycelial characteristic and occurred in segregating mycelial lines in the other strains. Colonies on malt agar, excepting the mycelial isolates, had only the slightest tinge of pink in the peripheral mycelium and the red pigment was often completely absent, the colony appearing brown.

Table 2. *Pigment colour range of* Phoma violacea. (*Observed during laboratory testing of paints*)

	Isolates		
	No. 2	No. 3	No. 4
Zinc oxide paint	FB 642/3	FB 642/3 VV 639/3	FB 642/3
White lead paint	FB 642/3 AP 618/3	FB 642/3 AP 618/3	VV 639/3 AP 618/3
Titanium dioxide paint	FB 642/3	FB 642/3	VV 639/3 AP 618/3

The colours are based on the Horticultural Colour Chart, British Colour Council, 1938.
FB, Flax blue: Ridgway Equivalent—light violet blue, 53*b*.
AP, Azalea pink: Ridgway Equivalent—nil.
VV, Veronica violet: Ridgway Equivalent—light violet, 59*b*.

The pigment has been observed inside the mycelium as lipid inclusions

(Massee, 1911; Nicot-Toulouse, 1953) and also as crystals in the surrounding medium (Bertel, 1904; Haensler, 1921). Both observations were confirmed, while it was also noted that crystals of a brown pigment formed in the malt agar medium on which isolates 1 and 3 were growing. When linseed oil was added to the malt medium, the pigment dissolved and stained the oil a characteristic red. Particular note was made of the production of pigment by isolates 4 and 61 and its solubility in oil, bearing in mind the origin of 61 (from emulsion paint on which there was no pigmentation) and 4 (human mouth): they are both considered to be *P. violacea* strains. The pigment in pycnidiaceous strains, if kept in the dark, becomes a blackish green in hyphae in agar.

The pigment, tested by shaking mycelial blocks in common solvents, was soluble in hot water, acetone, ethyl alcohol (95 %) and chloroform. In dilute mineral acids it formed red solutions, while in dilute alkalis its solubility varied from isolate to isolate, the pigment turning blue. Solutions of the pigment exposed to daylight for about a month became colourless. The constitution of the pigment was not investigated. Red anthroquinone pigments are fairly widespread in fungi and this particular pigment is possibly a compound related to phomazarin, a product of *Pyrenochaeta terrestris* that has been elucidated by Kögl, Wessem & Elsbach (1945).

PHOMA SAPROPHYTICA

The five isolates, 5 (Herb. I.M.I. 85471), 16 (Herb. I.M.I. 85470), 57*a*, 60*a* and 63, considered to represent a new species could easily be distinguished from *P. violacea* by the flat colony growth and wispy black aerial mycelium on malt agar. Pycnidia were abundant both at the surface and deep within the agar. The mycelium developed deeply in the agar and was jet black, as were the pycnidia. All isolates had a mean colony diam. of 90 mm. after 12 days' growth on malt agar at 25° C., a rate of growth greater than that of any of the *P. violacea* isolates. Other less definite characters included the larger spores and different pigmentation, which was consistently black, no red-violet colour ever being demonstrated. On Czapek-Dox agar the numerous pycnidia were matted together, forming above the surface with a slight aerial black mycelium, the whole giving a black mealy appearance. On cornmeal agar the black mycelium and pycnidia were mainly submerged. Isolate 60*a* came from the same site as 60 (*P. violacea* strain): each remained constant in culture and appeared to be a distinct species.

Phoma saprophytica sp.nov.

Saprophytica, colonia et pycnidiis nigris. Mycelium primo hyalinum, deinde griseum, conglobando nigram videtur. Pycnidiis nigra opaca, meristogenibus, subglobosa, ostiola saepe una, raro duabus vel tribus; superficialibus Czapek-Doxis autem immersis et frumentum agariis 50–300 (med. 110) μ. Colonia in hic mediis celeriter crescit; 90 mm. in 12 diabus, 25° C. Conidiophoris parva nec conspicua. Pycnosporis non solito extrusis; sine colore translucidis conglobando. Sporia non-septatis ellipsoideis hyalinis 3·8–9·9 × 1·9–4·1 (med. 6·1 × 3·1) μ. Non multum gelatinum liquescit non amplius 5 mm.

altitudine 42 diebus. Pigmentum in stratis oleo unctis non diffusum. Habitat. Saprophytica ex solo, pictis superficiebus, aëre, et caemento, nigra colonia. Typus. Ex solo, Exon, Anglia, 21.1.59, Herb. I.M.I. 85472.

Saprophytic. *Colony* and pycnidia black. Young mycelium hyaline quickly becoming translucent grey, and appearing black in mass. *Pycnidia* black, opaque, meristogenous, subglobose with usually one ostiole, occasionally 2–3; superficial on Czapek-Dox agar but immersed in malt and cornmeal agars; diam. 50–300 (av. 110) μ. Colony fast growing on the media listed above: 90 mm. in 12 days at 25° C. *Conidiophores* small and inconspicuous. *Spores* not generally extruded; colourless translucent in mass; non-septate, hyaline, ellipsoid, $3·8–9·9 \times 1·9–4·1$ (av. $6·1 \times 3·1$) μ. Weak liquefaction of gelatin (not exceeding 5 mm. in depth in 6 weeks). No diffusion of pigment on oily substrates.

Habitat. Saprophytic in soil on painted surfaces and cement, and in air. Type: from soil, Exeter, Devon, England, 12.1.59, Herb. I.M.I. 85472. Material has been deposited at the Botany and Plant Pathology Laboratory, Science Service, Ottawa; The Commonwealth Mycological Institute, Kew; and the Botany Department, University of Exeter.

The saprophytic nature of this species was established by performing inoculations similar to those carried out with *P. violacea*. No infections were obtained. With basic fuchsin uni- and bi-nucleate pycnospores were detected, also monokaryotic and heterokaryotic hyphae.

PHOMA spp.

There are several reports of paint films being 'attacked' by *Phoma* spp. which did not produce discoloration by the action of a pigment diffusing into the oil vehicle. Haensler (1921) describes a rather 'atypical' *Phoma* sp. and Rothwell (1958) and Drescher (1958) both record isolates of *P. glomerata* on oil and emulsion paints. Examination of a culture of this latter species and also other named *Phoma* spp. (Table 3) did not reveal any strains similar to *P. violacea* or *P. saprophytica*. *Phoma* spp. nos. 53*a* and 56 appeared atypical compared with all the other isolates. Neither produced a pink pigment and pathogenic studies revealed no parasitic

Table 3. *Comparative fungal cultures*

No.	Species	Source
10	*Pyrenochaeta terrestris* (Hansen) Gorenz *et al.*	CBS
11	*Macrophomina phaseoli* (Maubl.) Ashby (*Rhizoctonia bataticola* (Taub.) Butler)	CBS
12	*Leptosphaeria rubicunda* Rehm.	Herb. Univ. Sheffield, no. 1128 (J. Webster)
26	*Phoma solanicola* Prill. & Delacr.	NAAS, Starcross, Devon (W. Campbell)
41	*P. alternariaceum* Brooks & Searle	CMI (Herb. I.M.I. No. 17361)
42	*P. eupyrena* Sacc.	CMI (Herb. I.M.I. No. 45944)
43	*P. glomerata* (Cda.) Wollenw. & Hochapfel	CMI (Herb. I.M.I. No. 46259)
45	*Diplodina palmicola* Thüm.	CBS
64	*Pleospora betae* (Frank.) Bjorling	I.C.I., Jeallott's Hill
65	*Phoma lingam* (Tode) Desm.	I.C.I., Jeallott's Hill
	P. solanicola Prill. & Delacr. (nos. A2 (2), A6, T11 and T12.)	J. F. Malcolmson

Abbreviations see Table 1.

mode of life. They were considered as distinct saprophytic species occurring incidentally on paint. *Diplodina* sp. no. 57 was considered of similar status; it caused greater disfiguration of paintwork because of the production of a soluble red pigment.

DISCUSSION

Red-violet pigmentation is fairly common amongst the Sphaeropsidales and certain Ascomycetes, e.g. Gorenz, Larson & Walker (1949) report it in *Pyrenochaeta terrestris*; Koch & Rumbold (1921) in *Phoma insidiosa*; Webster (1957) in *Pleospora rubelloides*, *P. rubicunda* and *P. straminis*. Distinction between such pigmented parasitic species, in the imperfect state, and *Phoma violacea* could most easily be determined by pathogenic studies. Should the former have lost their pathogenicity, distinction would be extremely difficult to prove. However, none of the species mentioned in Table 3, including pigmented strains of *P. solanicola* (A2(2), A6 and T12), were found to be capable of growing on white lead paint under standard laboratory test conditions (Anon., 1952).

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CHITINOPHILIC CHYTRIDS FROM LAKE MUDS

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(With Plates 37 and 38 and 2 Text-figures)

Obelidium megarhizum sp.nov. and *Podochytrium chitinophilum* sp.nov. are described. *Phlyctochytrium aureliae* Ajello is a new British record. All three were obtained by baiting lake mud with chitin.

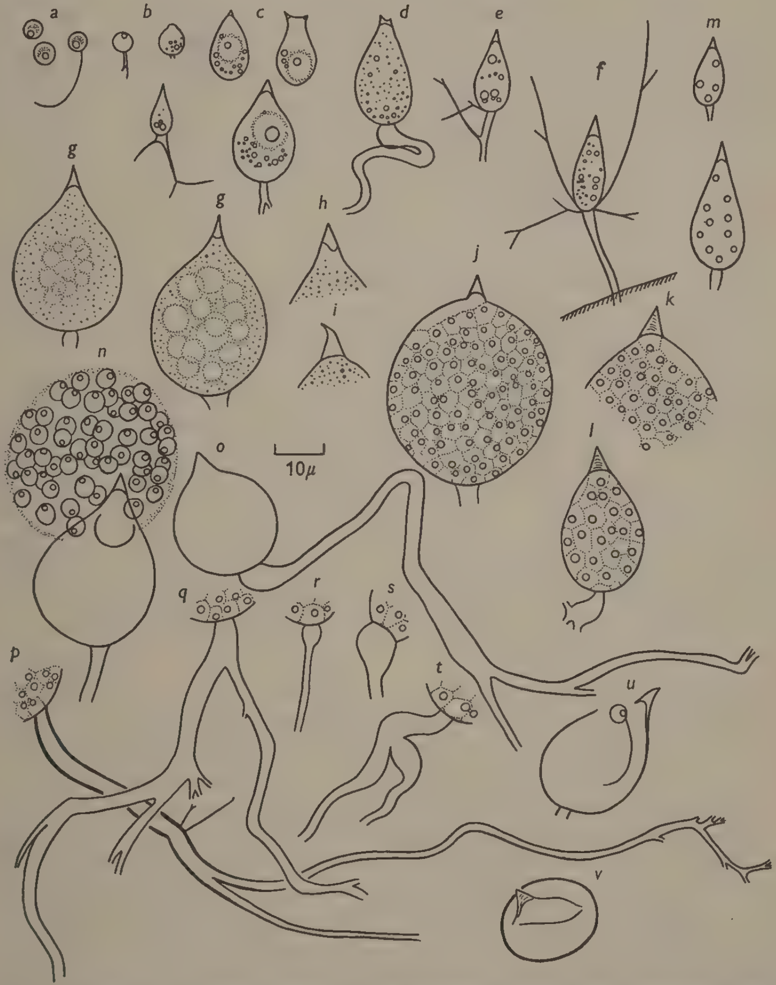
The species described here were obtained when submerged mud from the littoral zone of two bodies of fresh water was baited with termite wings, which have proved most useful as a chitin substratum, both in this and other work, since they require no pretreatment other than autoclaving, they float in the baiting dishes, and also provide a large surface area on which relatively uncontaminated growth of lower fungi takes place. The descriptions were made from the initial growth on the baits, as unifungal or bacteria-free cultures were not obtained.

Obelidium megarhizum sp.nov. was obtained from Malham Tarn, Yorks., England. After zoospore encystment the thallus soon develops a pointed apex (Text-fig. 1 *b*, *c*) which eventually becomes a solid spine. Specimens with two apical spines are noted occasionally (Text-fig. 1 *d*; Pl. 37 *a*). A characteristic later stage in development occurs when central vacuoles appear in the thallus (Text-fig. 1 *g*). At maturity sporangia are either approximately spherical (Text-fig. 1 *j*, *n*, *o*) or ellipsoid (Text-fig. 1 *m*). Dehiscence is inoperculate and involves liquefaction of a region of the sporangium wall near the spine. This region is clearly indicated in favourable cases, since there is a zone of clear cytoplasm immediately below it (Text-fig. 1 *j*–*l*). The zoospores emerge through a circular aperture and undergo collective swarming for up to 3 min. before they disperse (Text-fig. 1 *n*). Zoospores are spherical with a posterior lateral hyaline globule, clearly visible nuclear cap, and long posterior flagellum (Text-fig. 1 *a*). Once the sporangium is empty it becomes obvious that the apical spine is a solid structure (Text-fig. 1 *u*, *v*; Pl. 37 *e*).

There is a single rhizoidal insertion on the thallus and sometimes an apophysis (Text-fig. 1 *d*, *l*, *o*, *r*, *s*; Pl. 37 *a*, *b*, *d*). The remainder of the rhizoidal system consists of one or two long main broad trunks with very limited lateral branching. Normally both the apophysis (if present) and the rhizoids are in close contact with the substratum, the sporangium alone projecting above it (Pl. 37 *a*); but extramatrical rhizoidal systems have been seen occasionally (Text-fig. 1 *f*).

Clearly this chytrid must be placed in the Rhizidiaceae of Sparrow's (1943) classification. The nature of the rhizoidal system indicates the genus *Rhizidium*, but on the other hand the peculiar apical spine strongly suggests *Obelidium*. Since the spine occurs so consistently in the Malham

Tarn chytrid it seems advisable to regard this as the more significant character. It is noteworthy that in Sparrow's (1938) detailed account of *O. mucronatum* Nowakowski, the only currently accepted member of the genus, he recorded thalli with a tilted apical spine and thalli with two apical spines; and both of these minor variations from the typical condition have been noted in my material (Text-fig. 1 *i*, *d*). Differences from *O. mucronatum* are seen in the rhizoidal system, which is much less richly



Text-fig. 1. *Obelidium megarhizum* sp.nov. *a*, zoospores; *b*, young thalli; *c*, young thalli with central vacuole; *d*, young thallus with apophysis and two apical spines; *e*, young thallus; *f*, extramatrical thallus (substratum edge cross-hatched); *g*, later stages in development showing central vacuoles; *h*, *i*, apical spines in young thalli; *j–l*, mature sporangia showing zone of clear cytoplasm indicating where dehiscence will occur; *m*, ovoid mature sporangia; *n*, collective swarming at dehiscence; *o*, mature thallus outline showing extent of rhizoidal system; *p–t*, rhizoidal systems of mature thalli; *u*, zoospore emergence; *v*, surface view of dehisced sporangium showing relation of solid apical spine to the pore. All to the same scale.

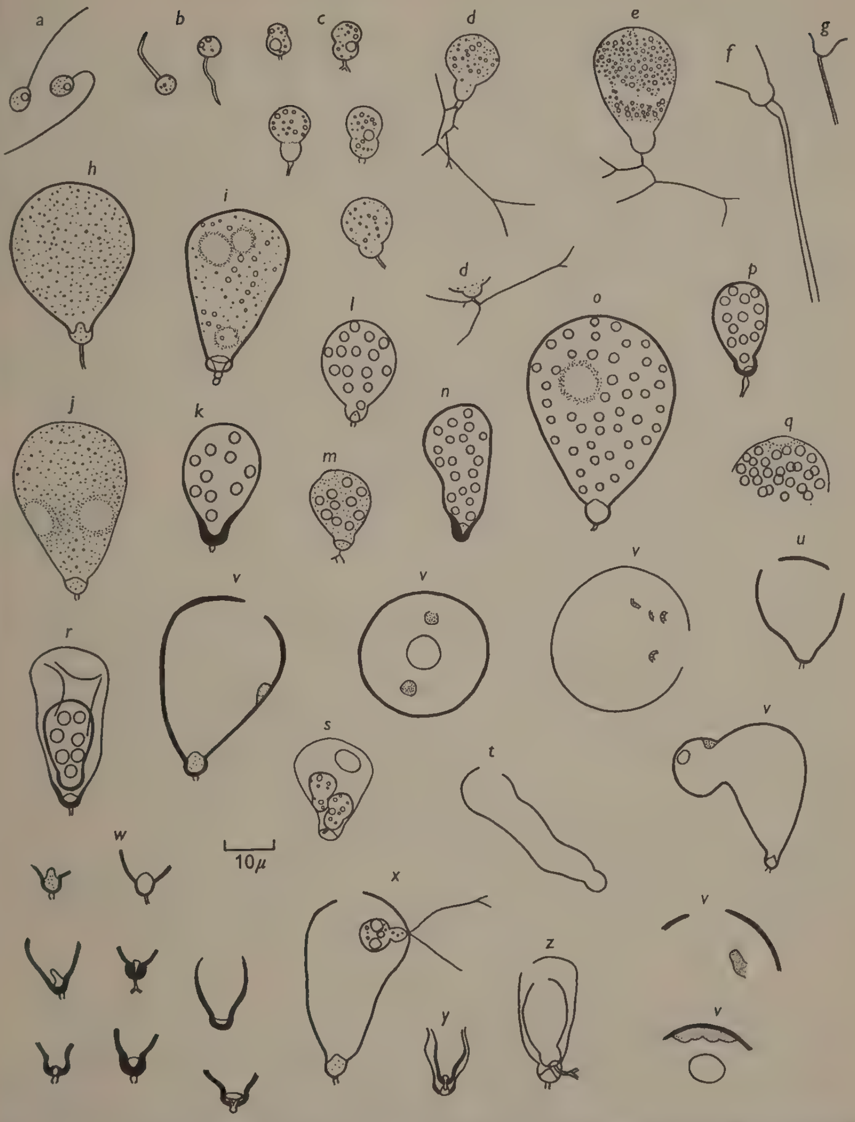
branched, and in the thin sporangium base perfectly continuous with the rest of the sporangium, rather than thickened and cup-like. It is apparent that a new species is involved.

Obelidium megarhizum sp.nov. (Text-fig. 1; Pl. 37)

Thallus monocentricus, eucarpicus, interbioticus, consistens ex globoso aut ovoide sporangio et systema rhizoidali quod ex sporangio in uno tantum loco oritur. Sporangia globosa 14·5–40 μ longa × 10–35 μ lata, aut ovoide 11·5–25 μ longa × 6–17 μ lata; cum solida spina apicali 2·5–8 μ longa × 1·5–6 μ lata in basi. Apophysis quoque 5·5–8·5 μ diam. nonnunquam adest. Reliquum systema rhizoidale consistit ex una aut duabus latis stirpibus principalibus, 1·5–5·5 μ diam., quae extenduntur ad 160 μ cum lateralibus ramis praecipue paucis. Zoosporae liberantur cum pars ex sporangii pariete prope spinam liquefit. Globosae sunt zoosporae 3·5–4·5 μ diam. cum una posteriore et laterali guttula olei hyalina et conformatione supra nucleum forma lunae crescentis et posteriore flagello longo. Emergunt una per foramen rotundum 5·5–15 μ diam. et inter se glomerant antequam seiunguntur. Sporas quiescentes non observavimus. Ex luto submerso ex Malham Tarn, Yorkshire, Anglia, esca 'chitin' fruens. Typus: Herb. I.M.I. 85111.

Thallus monocentric, eucarpic, interbiotic, consisting of a spherical or ovoid sporangium and a rhizoidal system arising from the sporangium at a single point. *Sporangia* spherical, 14·5–40 μ × 10–35 μ, or ovoid 11·5–25 μ long × 6–17 μ wide; with a solid apical spine 2·5–8 μ long × 1·5–6 μ wide at the base. An apophysis 5·5–8·5 μ diam. is sometimes present. The remainder of the rhizoidal system consists of one or two main broad trunks, 1·5–5·5 μ diam., which extend up to 160 μ with very limited lateral branching. *Zoospores* are released when a region of the sporangium wall near the spine liquefies; zoospores spherical, 3·5–4·5 μ diam., with a posterior lateral hyaline oil globule, nuclear cap, and long posterior flagellum; emerging together through a circular pore 5·5–15 μ diam. and swarming collectively before dispersing. *Resting spores* not observed. From submerged mud from Malham Tarn, Yorkshire, England, on termite wings. Typus: Herb. I.M.I. 85111.

Podochytrium chitinophilum sp.nov. was obtained from Wastwater (English Lake District). Initial development involves the formation of a delicate tubular rhizoid, and this has been observed when zoospores germinated inside dehisced sporangia (Text-fig. 2*b*). Subsequently the zoospore cyst expands unequally and the lower part remains unchanged in outline (Text-fig. 2*c–e*). Eventually, when the thallus is almost mature, a septum which cuts off the whole or part of this lower unchanged portion is usually visible (Text-fig. 1*h–j*; Pl. 38*g*). The tiny basal cell so formed remains sterile. In other individuals there is no septum but pronounced wall thickening at the sporangium base, and in still others both septum formation and wall thickening. Only very rarely have sporangia been seen with neither a septum nor basal thickening (Text-fig. 2*t*, *u*). The various conditions observed are illustrated in Text-fig. 2*h–p*, *r–z*, and Pl. 38*i–k*. Mature sporangia (Text-fig. 2*k–p*; Pl. 38*h*) are pyriform with the basal cyst; cylindrical or irregularly shaped ones occur occasionally (Text-fig. 2*t*, *v*). Dehiscence is inoperculate; a barely perceptible bulge with clear cytoplasm below it indicates the position of the dehiscence papilla, which is



Text-fig. 2. *Podochytrium chitinophilum* sp.nov. *a*, zoospores; *b*, germinating zoospores in a dehisced sporangium; *c*, young thalli; *d*, *e*, young thalli in which basal wall thickening or sterile cell formation has not yet developed; *f*, *g*, long unbranched rhizoids; *h–j*, young thalli with sterile basal cell; *k–p*, mature sporangia, *o*, with a dehiscence papilla in surface view; *q*, apex of mature sporangium showing dehiscence papilla; *r*, *s*, *x–z*, dehisced sporangia with zoospores which failed to emerge germinated *in situ*; *t*, dehisced sporangium; *u*, dehisced sporangium with two pores; *v*, dehisced sporangia showing wall nodulation (shaded); *w*, various types of basal wall thickening or sterile cell formation. All to the same scale.

apical or nearly so (Text-fig. 2*m*, *q*). Two dehiscence papillae occur very occasionally (Text-fig. 2*u*).

Although adequate material has been available, a sporangium has not been under observation at the precise moment when the zoospores emerged. They escape en masse through the pore and undergo brief collective swarming before dispersal. Zoospores are oval in motion with a posterior lateral hyaline globule and long posterior flagellum (Text-fig. 2*a*). Zoospores which fail to escape frequently germinate *in situ* (Text-fig. 2*r*, *s*, *x*–*z*), and in favourable cases it has been possible to trace the rhizoidal system of the daughter thallus through the wall of the parent (Text-fig. 2*x*, *z*). It is clear, therefore, that proliferation from the basal sterile cell is not involved.

Solid nodules of thickening have been observed on the walls of many dehisced sporangia (Text-fig. 2*v*; Pl. 38*k*), but are not invariably present. The rhizoid is always delicate and usually richly branched immediately below the sporangium (Text-fig. 2*d*, *e*), though it may extend up to 42 μ before branching (Text-fig. 2*f*, *g*). Mature sporangia are very easily detached from the rhizoidal system (Pl. 38*i*).

The Wastwater chytrid consists essentially of an epibiotic and pyriform sporangium with a basal cyst, and a delicate endobiotic rhizoid. This particular morphological form and relationship to the substratum is common in monocentric chytrids, and is exhibited by at least sixteen different described species in the genera *Chytridium*, *Phlyctidium*, *Podochytrium* and *Rhizophydium*. However, all these have been found on various algal and fungal hosts, with clear observational evidence of parasitism in most cases. The Wastwater chytrid, in contrast, is clearly saprophytic, and appears to be confined to chitin substrates. On the grounds of dehiscence mechanism and presence of a sterile basal cell, placing in the genus *Podochytrium* seems appropriate. The saprophytic habit and minute size of the sterile basal cell distinguish it sharply from other species of the genus, namely *P. clavatum* Pfitzer, recently described by Friedmann (1952), *P. cornutum* (Sparrow, 1951), *P. lanceolatum* (Sparrow, 1936), and *P. emmanuelense* (Sparr.) Sparrow & Paterson (1955). In the present state of our knowledge it appears advisable to describe the Wastwater chytrid as a new species.

Podochytrium chitinophilum sp.nov. (Text-fig. 2; Pl. 38)

Thallus monocentricus, eucarpicus, consistens ex epibiotico sporangio pyriformi, cum angusta basi (haec principio fuit pars zoosporae quae non se pandit) et uno tantum rhizoido tenui et ramoso et endobiotico. Sporangia 12·5–40 μ longa × 9–30 μ lata, et angusta basis 3–5 μ lata. Sporangia cum sterili in basi cella, 0·5–5 μ longa × 1–4·5 μ lata, aut in basi crassata, aut cum his ambabus formis. Sporangia quae nec sterilem in basi cellam habent nec sunt in basi crassata, perraro tantum inveniuntur. In parte sporangii superiore, tumores solidi interdum in pariete adsunt usque ad 12·5 μ longi. Zoosporae liberantur cum papilla quaedam, vix bene in apice sporangii visa, liquefit. Nonnunquam fieri potest ut duae papillae adsunt, raro tamen. Zoosporae, quae emergunt per foramen 3·5–7·5 μ diam. sunt forma ovata dum se movent et 4–5 μ × 3·5–4·5 μ cum una posteriore et laterali guttula olei hyalina et longo flagello posteriore. Emergunt una et inter se glomerant paulisper antequam seiunguntur. Sporas quiescentes non observavimus. Ex luto submerso ex Wastwater, Cumberland, Anglia, esca 'chitin' fruens. Typus: **Herb. I.M.I. 85112.**

Thallus monocentric, eucarpic, consisting of an epibiotic pyriform sporangium with a basal cyst, the latter derived from an unexpanded part of the zoospore, and a single, delicate, branched, endobiotic rhizoid. *Sporangia* 12·5–40 μ long × 9–30 μ wide, basal cyst 3–5 μ across. Sporangia with a sterile basal cell, 0·5–5 μ long × 1–4·5 μ wide, or with basal thickening, or both. Sporangia without either a sterile basal cell or basal thickening are found only rarely. In the upper part of the sporangium solid nodules up to 12·5 μ long may be present on the wall. *Zoospores* are released when a barely visible papilla, occasionally two, at the apex of the sporangium liquefies; they emerge through a pore 3·5–7·5 μ diam. and are oval in motion, 4–5 μ × 3·5–4·5 μ, with a posterior lateral hyaline oil globule and long posterior flagellum; they emerge together and swarm collectively for a short while before they disperse. *Resting spores* not observed. From submerged mud from Wastwater, Cumberland, England, on termite wings. Typus: Herb. I.M.I. 85112.

Growing in close association with *P. chitinophilum* was *Phlyctochytrium aureliae* Ajello (1945), which occurred on grass leaf baits in addition to termite wings. Sporangia (Pl. 38*i*, *l–n*) were 12–20 μ diam. and ornamented with bipartite teeth arranged in 4–6 concentric whorls. Thread-like proliferations of the apices of the teeth (described by Ajello) were frequently observed in the Wastwater material.

My thanks are due to Prof. C. T. Ingold for suggesting the use of termite wings as bait, to my colleague Mr K. E. Marshall for collecting the Malham Tarn mud sample, to Miss P. J. Townley for technical assistance, and to Mr W. J. Downer for kindly providing the Latin diagnoses.

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EXPLANATION OF PLATES 37 AND 38

PLATE 37

Obelidium megarhizum sp.nov. *a*, Young thallus with two spines at the apex, and mature sporangium, both with a typical rhizoidal system; *b*, young thalli; *c*, mature sporangium; *d*, young thalli and mature sporangium; *e*, *f*, dehisced sporangia at two levels of focus, two zoospores which failed to emerge being visible. All × 1000.

PLATE 38

g–k, *Podochytrium chitinophilum* sp.nov. *i*, *l–n*, *Phlyctochytrium aureliae* Ajello. *g*, Young thallus; *h*, mature sporangium; *i*, detached dehisced sporangium with thallus of *Phlyctochytrium aureliae* below; *j*, *k*, dehisced sporangia, *k* shows wall nodulation near the apex; *l*, *m*, surface views of dehisced sporangia; *n*, optical section of dehisced sporangium. All × 1000.

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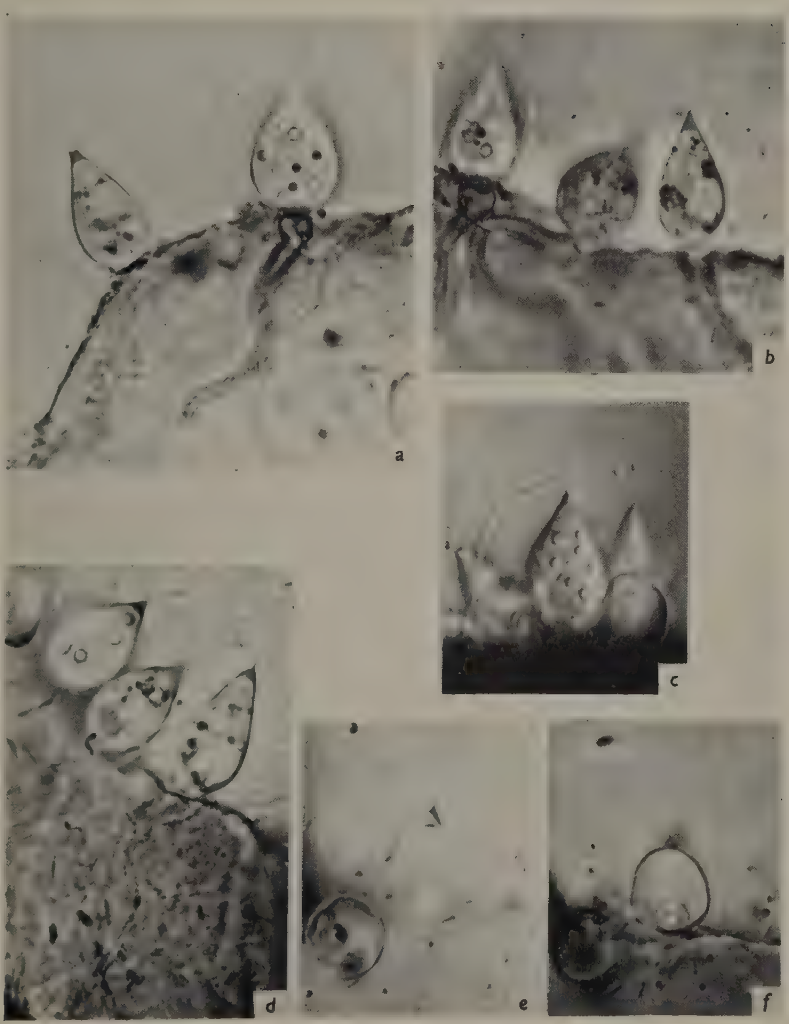
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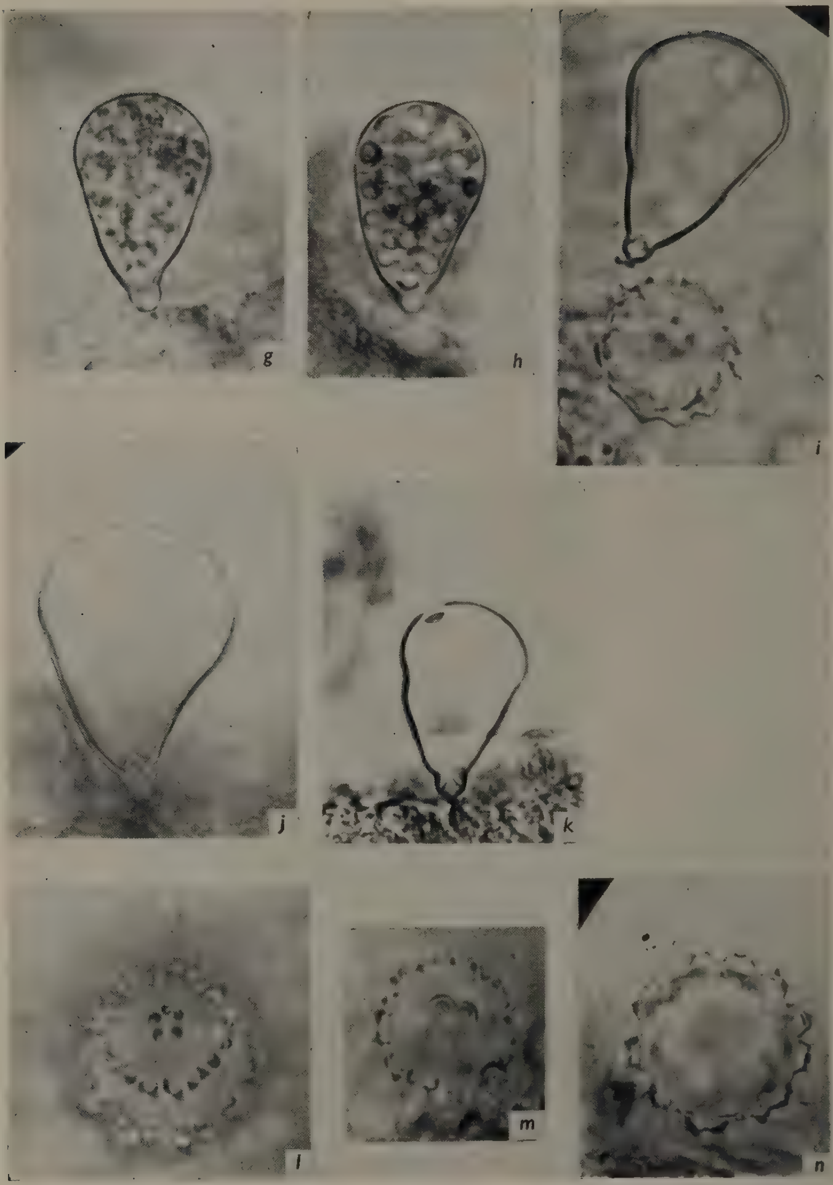
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SPORE LOAD, LIGHT INTENSITY AND PLANT NUTRITION AS FACTORS INFLUENCING THE INCIDENCE OF CLUB ROOT OF BRASSICAE

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Light intensity markedly affects the relationship between spore load and number of plants developing club root (or their disease index). A direct relationship exists at low light intensities up to a high level of spore load, but at a high light intensity this relationship exists only at fairly low spore loads.

At any spore load within the range 10^1–10^7 spores/g. soil, the disease index for plants was lowest at the lowest light intensity employed. At high spore loads the number of diseased plants is not influenced by light intensity, but at low spore loads the highest number of diseased plants is associated with the highest light intensity.

In composts with different spore loads, the number of diseased plants was shown to be uninfluenced by the supply of nitrogen, potassium or phosphorus. Plants well supplied with nitrogen had a lower disease index than had those not receiving more nitrogen than was adequate for very slow growth. This nitrogen effect was usually more marked as the fertilizer dosage increased.

The bearing of these findings on the use of a pot test for evaluation of spore loads of *P. brassicae* in soils is discussed.

It has been observed (Bremer, Wehnelt & Brandenburg, 1937; Colhoun, 1953) that, even when other conditions are equally favourable for infection and for development of the disease, less severe attacks of club root occur in glasshouse experiments during winter than in summer. Data obtained later in a study of the relationship between spore load and severity of club root suggested that variations in spore load might not exercise the same effects when the growing conditions vary, this being particularly true in relation to light intensity (Colhoun, 1957).

There is lack of agreement between previous workers who have studied the incidence of club root in relation to spore load. No direct relationship was found between spore load and number of diseased plants by Naumov (1928) or Gibbs (1931), but Bremer *et al.* (1937) and Macfarlane (1952) disagreed with this conclusion. The reason for such disagreement might be attributed to variation in such factors as soil reaction, moisture or infectivity of the inoculum, but no evidence exists indicating the part played by light intensity.

Although some attention has been given to the effects of mineral nutrition on the incidence of club root, no exact information exists regarding its influence in relation to other factors. It was therefore decided to study the influence of mineral nutrition on the disease, with particular reference to spore load.

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METHODS

Many of the methods employed have already been described. Cabbage plants of the variety Enfield Market were used throughout the work. These were raised in sterilized compost; normally the seed boxes were maintained in the open air but, in experiments involving light intensity as a factor, the seedlings were raised in glasshouses under the appropriate level of lighting. About 4 weeks after sowing they were transplanted into contaminated soil, twenty seedlings being planted in each pot.

Artificially inoculated soil or compost was used, the inoculum being collected from diseased cabbage plants and stored for very short periods at −3° C. To maintain uniformity, the spore load is expressed as the equivalent of spores/g. oven-dried soil or compost. A light loam from one source was used, except in experiments involving nutrition when a compost was prepared by mixing equal quantities of poor soil, sand and horticultural peat, with which various doses of fertilizers were incorporated. All combinations of nitrogen (N), potassium (K) and phosphorus (P), supplied as sulphate of ammonia (20·6 % N), sulphate of potash (48·6 % K_2O) and superphosphate (18·5 % soluble P_2O_5), were compared with untreated compost. Fertilizers were supplied at the following dosages in g./pot, each of which contained the equivalent of 3 kg. of oven-dry compost

	Level 1	Level 2	Level 3
N	0·5	3·0	6·0
K	0·5	3·0	6·0
P	1·5	9·0	18·0

Ground limestone at the rate of 2·5 parts to 1000 parts by weight of compost was added to all pots in nutrition experiments. The moisture content of contaminated soil or compost contained in water-tight tins was adjusted to 70 % of the soil's water-holding capacity, this level of moisture being maintained during the period of plant growth by watering on alternate days.

All the plants growing in contaminated soil or compost were maintained in heated glasshouses. In experiments where light was considered as a factor, the three following levels of light intensity were provided except during winter: (1) normal light, (2) half normal light, secured by shading the plants with galvanized iron sheets with circular perforations, (3) quarter normal light, provided by shading with perforated galvanized iron sheets covered with fine muslin. During winter, the lower light level was provided by normal daylight and the higher level by using high-pressure mercury-vapour lamps to supplement daylight from 9 a.m. to 9 p.m. Measurements of light intensity were made, using a Weston meter, the comparisons being based on readings made between noon and 2 p.m. When the effects of light intensity were being studied, the seedlings were maintained under the same conditions during the entire period of growth.

The plants were uprooted usually after 4 weeks' growth from transplanting, and the percentage showing symptoms of club root visible to the naked eye was calculated for each pot. The diseased plants were divided into four subgroups according to the position and size of the clubs:

(1) very slight, (2) slight, (3) moderate, and (4) very severe, often with clubbing of the hypocotyl. From these values, the disease index was calculated for each pot. Unless otherwise stated, all mean values are based on data for four pots per treatment. Observations on the growth of plants were made at the end of each experiment, and in some instances the fresh weight of the plants in each pot was determined.

RESULTS

Effects of light intensity and spore load

Experiment 1. The seedlings were raised in John Innes seed compost and transplanted into contaminated soil of pH 5·0, which was well supplied with nitrogen, potassium and phosphorus. The period of growth in contaminated soil was from 6 September to 15 October 1957, during which time the mean air temperature was 23·9° C. Six spore loads, varying from 10^0 to 10^6 spores/g. soil, and three levels of light intensity were employed. Two pots constituted a unit for each treatment.

The results (Table 1) show that the disease index increased with spore load up to 10^3 spores/g. soil under full-light intensity, but at the lower light intensities the index increased with spore load up to its highest value. Percentage number of diseased plants increased under full-light intensity with spore load up to 10^2 spores/g. soil, but at the lower light intensities the direct relationship between spore load and number of diseased plants was maintained up to much higher spore loads. Similar values for percentage number of diseased plants or for the disease index were recorded at (1) full-light intensity and spore load of 10^2, (2) half-light intensity and spore load of 10^4, (3) quarter-light intensity and spore load of 10^6. At all spore loads the disease index was highest when the light intensity was greatest, but at high spore loads the number of diseased plants was not influenced by lighting.

Table 1. *Effects of light intensity and spore load*

Mean % diseased plants and disease index.*

Spore load	Full intensity of autumn light	Half intensity of autumn light	Quarter intensity of autumn light
10^0	42 (104)	0 (0)	0 (0)
10^1	51 (122)	3 (9)	2 (3)
10^2	92 (271)	13 (34)	23 (30)
10^3	100 (373)	79 (232)	56 (125)
10^4	100 (384)	92 (280)	91 (209)
10^6	98 (385)	100 (314)	98 (296)

* Mean values for disease index are given in parentheses.

Experiment 2. This experiment was carried out during winter, the plants being grown in contaminated soil from 13 January to 18 February 1958 with a mean air temperature of 22·4° C. Six levels of spore load, varying from 10^1 to 10^7 spores/g. soil, and two levels of light intensity were employed. The higher light intensity was somewhat lower at mid-day than the highest level provided by normal autumn light in Exp. 1. Each treatment was represented by two pots.

The results (Table 2) show that at both light intensities the disease index increased with spore load up to the highest level, whereas percentage number of diseased plants increased with increasing spore load at all but the highest levels. At all spore loads the disease index was higher at the higher light intensity. Although the number of diseased plants was again uninfluenced by light intensity at the highest spore loads, yet at low or intermediate levels of spore load more plants were diseased at the higher light intensity.

Table 2. *Effects of light intensity and spore load*

Mean % diseased plants and disease index.*

Spore load	Winter light supplemented by artificial lighting	Winter light without supplementation
10^1	32 (59)	0 (0)
10^2	48 (116)	8 (13)
10^3	62 (175)	14 (26)
10^4	89 (252)	75 (144)
10^6	95 (298)	100 (246)
10^7	100 (365)	95 (266)

* Figures within parentheses.

Effects of plant nutrition and spore load

Experiment 3. Seedlings were raised in compost, containing various combinations and levels of N, K and P, and were then transplanted into contaminated compost of the same composition as the seed-bed. The spore load employed was 10^3 spores/g. compost. The plants grew in the contaminated compost from 17 June to 17 July 1957, the mean air temperature being 23·2° C. The results are presented in Table 3.

Table 3. *Effects of mineral nutrition at low spore load*

Mean % diseased plants and disease index.*

	Level 1	Level 2	Level 3
N	100 (301)	95 (241)	100 (237)
K	100 (310)	99 (317)	99 (316)
P	100 (293)	98 (286)	100 (311)
NK	93 (285)	93 (226)	90 (209)
NP	96 (222)	94 (222)	89 (202)
KP	100 (334)	99 (286)	98 (292)
NKP	99 (289)	98 (291)	93 (209)
Untreated	96 (350)	99 (321)	96 (301)

* Figures within parentheses.

Using angular transformation of the percentage number of diseased plants in each pot, analysis of variance was applied to the data but no significant effects associated with treatment were demonstrated. When the data for disease index were considered, there was a significant effect of nitrogen at all levels (levels 1 and 3, $P < 0{\cdot}001$; level 2, $P < 0{\cdot}01$), while at level 1 there was a significant effect of phosphate ($P < 0{\cdot}05$) and the PK interaction was also significant ($P < 0{\cdot}005$). The magnitude of the nitrogen effect is shown in Table 4.

Table 4. *Effect of treatment with nitrogen on mean values for disease index*

	Level 1	Level 2	Level 3
N	274	245	214
No N	322	303	305
S.E. of above means	±8·4	±13·9	±14·2

Table 5. *Effects of mineral nutrition at high spore load*

Mean % diseased plants and disease index.*

	Level 1	Level 2	Level 3
N	100 (376)	100 (361)	97 (239)
K	100 (372)	100 (384)	100 (384)
P	100 (389)	100 (395)	100 (380)
NK	100 (369)	100 (348)	98 (314)
NP	100 (368)	100 (369)	99 (349)
PK	100 (386)	100 (385)	100 (351)
NPK	99 (386)	100 (390)	100 (394)
Untreated	96 (354)	100 (372)	100 (383)

* Figures within parentheses.

Experiment 4. This experiment differed from Exp. 3 only in that spore load was increased to 10^6 spores/g. compost. The period of growth in the contaminated compost was from 23 April to 16 May 1957, when the mean air temperature was 23·1° C.

The results given in Table 5 show that percentage number of diseased plants was not influenced by nutrition. Considering the data for disease index, significant nitrogen effects were demonstrated at level 2 ($P < 0{\cdot}025$) and at level 3 ($P < 0{\cdot}001$). The phosphate effect was significant at level 2 ($P < 0{\cdot}025$) and at level 3 ($P < 0{\cdot}001$), while at level 3 a significant potash effect ($P < 0{\cdot}025$) was obtained, the NK and NP interactions being also significant ($P < 0{\cdot}001$).

At the end of the experiment, the fresh weight of the plants in each pot was recorded. These values and the corresponding ones for disease index were compared but no relationship between the two sets was found; quite high values for disease index were often associated with poor growth of the plants.

Experiment 5. Seedlings were raised in John Innes seed compost and transplanted into artificially contaminated composts containing three levels and all combinations of N, K and P. All the plants had therefore been well supplied with N, K and P up to the time of transplanting. The growth period in the contaminated compost was from 8 August to 13 September 1956, when the mean air temperature was 19·5° C. The spore load was 10^6 spores/g. compost. Two pots constituted a unit for each treatment.

Analysis of variance was applied to the data obtained for each level of treatment. The disease index was significantly reduced by nitrogen and by phosphate at each level. Nitrogen effect: level 1, $P < 0{\cdot}01$; level 2, $P < 0{\cdot}005$; level 3, $P < 0{\cdot}001$. Phosphate effect: level 1, $P < 0{\cdot}01$; levels 2 and 3, $P < 0{\cdot}025$. At level 2, the NK interaction was significant ($P < 0{\cdot}05$). Using transformed values for the percentage number of diseased plants in each pot, no effects of treatment were found.

Experiments 6 *and* 7. Exp. 6 constituted a replica of that portion of Exp. 5 in which fertilizers were applied at level 3, except that four pots constituted a unit for each treatment. Exp. 7 differed from Exp. 6 only in that the spore load was 10^3 spores/g. compost. In Exp. 7 the spores were from very young clubs on young plants grown in a glasshouse, whereas the spores used in all other experiments were from mature plants grown under field conditions. The duration of plant growth in the contaminated compost was as follows: Exp. 6, 23 April to 16 May 1957 (mean air temperature 23·1° C.); Exp. 7, 1 July to 1 August 1957 (mean air temperature 22·0° C.).

Statistical analysis of the data obtained in the two experiments showed that in each instance nitrogen significantly reduced the disease index (Exp. 6, $P < 0{\cdot}005$; Exp. 7, $P < 0{\cdot}001$) whereas other treatments produced no significant effects. The percentage number of diseased plants was not influenced by any treatment.

Very low values for number of diseased plants and for disease index were recorded in Exp. 7. Since all other conditions were favourable for infection and development of the disease, it may be suggested that infection did not readily occur when spores from young clubs were employed.

Comparison of the data obtained in Exp. 6 for fresh weight of plants in each pot and for disease index did not suggest the existence of any relationship between these sets of values.

DISCUSSION

Club root can be controlled by liming only if the spore load is low and therefore when growers are being advised regarding liming it is desirable that an estimate of the spore load should be available. By the application of a pot technique (Colhoun, 1957), it is possible to determine if a given area is contaminated with *P. brassicae* and, provided caution is observed, the spore load may be evaluated within wide limits. The results now presented demonstrate clearly that the light intensity under which plants are grown must be carefully considered when evaluations of spore load are being made. For example, if a low value for number of diseased plants or disease index is obtained when plants are grown under a high light intensity, this may indicate a much lower spore load than a similar value obtained for plants growing under a low light intensity. This difference is due to the fact that at low light intensities there is a direct relationship between spore load and either disease index or number of diseased plants within a wide range of spore loads, whereas with a high light intensity this relationship exists only at low levels of spore load.

It may be expected that if plants of the same age are subject to the same level of infection at the same time, those showing the highest rate of growth should produce the largest clubs. During the present work, however, no clear relationship has been found to exist between the size of plants and either the number showing clubs or the disease index, which involves the number of diseased plants in a population as well as the size and position of the clubs. It cannot, therefore, be suggested that plants growing under a high light intensity show a higher value for disease index, or for the number of clubbed plants, than do those under a low light intensity because of differences in growth rate. The possibility may perhaps exist that light intensity may influence root diffusates, which in turn may have an effect on spore germination and infection.

It has been consistently demonstrated during this investigation that the number of diseased plants is not influenced by the supply of nitrogen, phosphorus or potassium. This is in disagreement with the conclusions reached by Pryor (1940), who found in experiments with sand cultures that more clubbed plants of susceptible hosts occurred in the presence of an abundance of nitrogen or potassium or when nitrogen was deficient, comparisons being made with a complete nutrient solution sufficient to maintain moderate growth of the plants. It does not appear that this disagreement can be attributed to differences in the spore load used in the two investigations.

The results now presented show that when all other treatments are neglected, the disease index for plants receiving nitrogen is usually less than that for those not receiving more nitrogen than is adequate to promote very slow growth. This nitrogen effect was usually more marked as the dosage with fertilizers was increased. It was demonstrated at both high and low spore loads, when comparisons were made between plants well supplied with nitrogen and those in which nitrogen was deficient throughout growth. When all plants received abundant nutrients up to transplanting time but variations in the nutrients supplied were then introduced, the nitrogen effect was apparent if comparisons were made between plants receiving little nitrogen and those receiving abundant nitrogen after transplanting. Throughout the investigation, no consistent effects of potassium or phosphorus were demonstrated. In some experiments phosphorus significantly increased the disease index, whereas in others it brought about a reduction. In considering these results it may be borne in mind that Walker & Hooker (1945) found that although a deficiency of nitrogen or potassium increased the disease index of plants in water culture, a similar effect was brought about by the provision of an excess of either of these elements. Palm (1958) also found that initial infection and development of clubs were stimulated by increases of potassium in nutrient solutions.

It is obvious from the results now presented that when soils are being tested for the presence of *P. brassicae* varying fertility levels will not influence the number of diseased plants, at least when the spore load is within the range 10^3–10^6 spores/g. soil. Variations in nitrogen content may, however, influence the disease index to some extent. Gwynne (1959) has pointed out that trouble with nutritional disorders when testing soils for the presence of *P. brassicae* may be obviated by analysing the soil samples, so that any major element that is deficient can be added.

When the effects of spore load are being studied in artificially contaminated soils, the maturity of spores is a factor which must be considered. Some spores from recently formed clubs are certainly capable of germinating and bringing about infection but, as has also been found by Macfarlane (1952), mature spores are more effective than young spores in causing club root.

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INDUCED MUTATION FOR PATHOGENICITY IN *PUCCINIA CORONATA AVENAE*

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(With 1 Text-figure)

The production of a mutant from *Puccinia coronata avenae*, race 96, by ultra-violet irradiation is reported. The mutant differs from the parent race in its ability to attack cultivars of a differential host series resistant to race 96, and in being avirulent to one cultivar susceptible to race 96. The mutant is identical in reaction on the differential host series to race 277 reported independently from Argentina and Israel.

The significance of mutation to the evolution of new physiological races is discussed. The preliminary results appear to be contrary to the theory of complementary gene action in host and parasite for this particular rust species.

It has long been known that within species of the rust fungi, there are forms or races morphologically indistinguishable but differing from each other in one or more physiological characteristics. Economically, by far the most important of these is pathogenicity, and workers concerned with cereal rust fungi have concentrated on pathogenic specialization and the ability of the various biologic forms to infect different cultivars of the host species. In *Puccinia coronata* Corda f.sp. *avenae*, the oat crown rust fungus, physiologic specialization is very highly developed, and cereal breeders and plant pathologists have been concerned with the occurrence, prevalence and mode of origin of these races (Frenzel, 1930; Murphy, 1935; Straib, 1937; Griffiths, 1958).

It has been demonstrated that new races of rust pathogens may arise through sexual hybridization (Newton, Johnson & Brown, 1930), heterokaryosis (Nelson, Wilcoxson & Christensen, 1955; Nelson, 1956; Watson, 1957*a*), and by mutation. Johnson & Newton (1946) regard hybridization as a mechanism leading to reassortment of genetic factors, but point out that the origin and diversity of these factors is dependent on mutation. Spontaneous mutation at loci controlling the pathogenic reaction has been demonstrated in *P. graminis tritici* (Stakman, Levine & Cotter, 1930; Newton & Johnson, 1939; Watson, 1957*b*), *P. glumarum tritici* (Gassner & Straib, 1932), *P. triticina* (Roberts, 1936), and *P. anomala* (D'Oliveira, 1939). It is extremely probable that there are many further instances of spontaneous mutation for pathogenicity in rust fungi, but that these have been overlooked due to the difficulty of differentiating, with certainty, mutants from possible contaminants. Recently, a large number of induced mutations from avirulence to virulence has been reported for *Melampsora lini* by Flor, who used ultraviolet irradiation (Flor, 1956) and X-rays (Flor, 1958). Flor (1960) demonstrated that these mutations to virulence were recessive to the corresponding wild-type avirulence alleles, the pattern of inheritance supporting his earlier-formulated hypothesis of a gene-for-gene relationship between rust reaction in the flax host and pathogenicity in the parasite (Flor, 1955).

Puccinia coronata avenae is a heteroecious, long-cycle rust capable of developing new pathogenic forms through recombination on its alternate host, *Rhamnus catharticus*. There is, however, no reported evidence of new races having arisen as a result of heterokaryosis and only one record of mutation (Williams—cited by Harland, 1955). Schwinghamer (1958), in discussing the effect of various sources of radiation on several rust species (including *Puccinia coronata avenae*) has calculated from the kinetic data the sensitivity and number of targets at sites controlling the pathogenic reaction, but there appears to be no record in the literature of any further study of induced mutants in this rust. The purpose of this paper is to present some results obtained from an irradiation experiment with race 96 of *P. coronata avenae*.

MATERIALS AND METHODS

Irradiation treatments

After repeated inoculation tests on a series of differential host cultivars, uredospores of *P. coronata avenae* race 96, derived originally from a single spore isolate, were exposed to radiation from a 15 W. ultraviolet lamp. The uredospores, previously dried for 3 days in a desiccator containing calcium chloride, were spread as a thin film over smooth cards and placed at a distance of 7 in. from the lamp. The first set of irradiated uredospores was utilized entirely to determine the optimum exposure required to give 5–10 % survival. Two cards were removed from the lamp at intervals of 2, 4, 8, 16, 32, 64 and 128 min., and spores from each card were spread over the surface of a Petri dish containing 3 % sucrose agar. Two cards of unirradiated spores were treated similarly and served as controls. Germination counts of 1000 spores in each dish were made 24 hr. after irradiation. According to Schwinghamer (1958), dosage/survival curves obtained from measurements of spore germination on agar are similar to those obtained from host infection tests when ultraviolet is used as the radiation source. Hence our data (Fig. 1) were taken to indicate that a dosage of about 90 min. would yield some 5–10 % survivors from the viable spore population, and this dosage was therefore adopted for subsequent experiments.

Inoculation tests

Seedlings of the host cultivars Bond, Ascencao, Victoria and Hawkeye, all with proven resistance to race 96, together with the susceptible cultivar S. 172, were inoculated in the first leaf stage with the treated uredospores immediately after irradiation. A similar series of seedlings was inoculated with unirradiated race 96 from the same original uredospore population. Special precautions were taken throughout the investigation to prevent contamination. All plants were sown, grown and prepared for inoculation in a separate glasshouse where there were no infected plants, and, as a further precaution, the pots were incubated under glass chimneys topped with glass sheets after inoculation.

The inoculum was applied with a flame-sterilized scalpel, running it over the surface of the leaves in such a way as to ensure an abundant and uniform spread of spores. In this way a spore load of approximately 5000 was applied to each fully extended leaf of about 3–4 in. in length. About forty seedlings of each cultivar were treated in this way with irradiated uredospores, and five seedlings with unirradiated uredospores of race 96 as control.

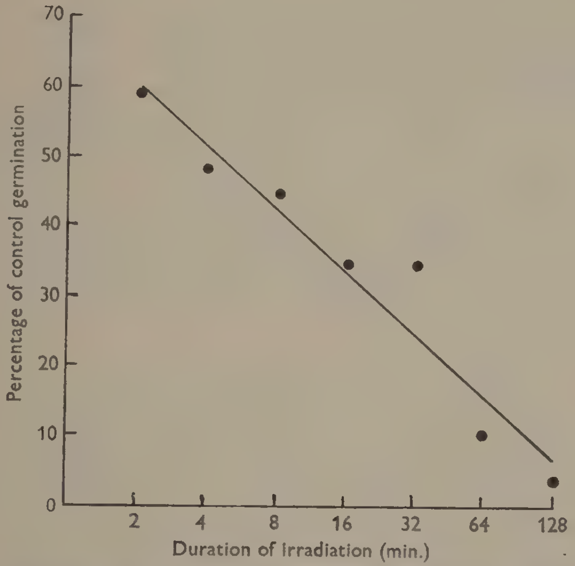

Fig. 1. Germination on 3 % sucrose agar of ultraviolet-irradiated uredospores of *Puccinia coronata avenae*, race 96

In subsequent experiments involving inoculation of the standard set of differential oat cultivars, ten or more seedlings of each were used for each inoculation; the recording of the reaction followed the classification given below:

Host reaction	Symbol and infection type
Highly resistant	0 = No uredospore pustules developed. Necrotic or chlorotic flecks usually present
Resistant	1 = Occasional, very minute pustules accompanied by pronounced necrosis. Necrotic areas produced without development of pustules
Moderately resistant	2 = Pustules small and surrounded by necrotic or markedly chlorotic areas
Moderately susceptible	3 = Pustules fairly abundant, medium sized and surrounded by chlorotic areas. Necrotic areas entirely absent
Very susceptible	4 = Pustules abundant and large, showing copious production of uredospores. No necrosis and normally no chlorosis

All possible efforts were made to conduct the tests under conditions as near as practicable to those prescribed and recommended for race identification work. In the first tests when resistant or non-typical reactions were obtained, repeated inoculations were performed.

EXPERIMENTAL RESULTS

Primary inoculation results

Uredospore pustules appeared on the S. 172 plants inoculated with unirradiated spores 11 days after inoculation, but no signs of sporulation were evident on the plants inoculated with ultraviolet-treated uredospores until 20 days had elapsed, when twenty-five separate pustules were recorded on the susceptible host cultivar and one uredospore pustule on Landhafer (Table 1). When the primary uredospore pustules were fully developed, further inoculations were undertaken to provide sufficient spore material for subsequent tests on the standard set of differential host cultivars. Separate cultures were established from each of the twenty-six pustules derived from irradiated uredospores; the twenty-five formed on S. 172 (cultures 1–25) were propagated on seedlings of the same cultivar, and the single pustule on Landhafer (culture 96/M-1) was continued on Landhafer seedlings.

Infection reactions on the differential cultivars

The results of inoculating ten or more seedlings of ten differential host cultivars with race 96, with cultures 1–25 and with culture 96/M-1, respectively, are given in Table 2. All twenty-five cultures established on S. 172 from irradiated spores gave infection recordings similar to those of race 96, but culture 96/M-1 (developed on Landhafer) gave an entirely different pathogenicity spectrum. It was clearly pathogenic on six host cultivars which were resistant to race 96, and accordingly must be regarded as a mutant race with its own distinctive pathogenic reaction. It is of interest to note that although the cultivars Anthony, Appler, Landhafer, Santa Fé, Trispernia and Bondvic were susceptible to the new race (indicating a mutation from avirulence to virulence in the pathogen), the position was reversed with the diploid cultivar Saia which, though susceptible to race 96, proved to be resistant to culture 96/M-1. It has now been established that the pathogenicity spectrum of the mutant is identical with that of race 277, first discovered in Argentina by Vallega and Cenoz in 1954 (Simons, 1955) and subsequently reported again in Israel by Wahl (1958). Another pathogenic feature of this mutant and of the naturally occurring race 277 is that they differ sharply from most races attacking Landhafer in their inability to parasitize the cultivar Bond.

DISCUSSION

As previously pointed out, sexual hybridization and mechanisms such as heterokaryosis and the parasexual cycle provide a means whereby the reassortment of genetic factors can take place, frequently leading to the evolution of new physiologic races. All these mechanisms, however,

Table 1. *Results of primary inoculations with ultraviolet-irradiated uredospores of* Puccinia coronata avenae *race* 96

Host variety	Irradiated uredospores		Control race 96	
	No. seedlings inoculated	No. pustules formed	No. seedlings inoculated	No. pustules formed
S. 172 (susceptible)	19	25	5	*c.* 200
Ascencao	55	0	5	0
Bond	57	0	5	0
Hawkeye	37	0	5	0
Landhafer	46	1	5	0
Victoria	46	0	5	0

Table 2. *Infection types produced on the standard set of ten differential host cultivars by twenty-six cultures established from irradiated uredospores of* Puccinia coronata avenae *race* 96

Uredospore cultures	Differential host cultivars									
	Anthony	Victoria	Appler	Bond	Landhafer	Santa Fé	Ukraine	Trispernia	Bondvic	Saia
Race 96 (control)	1 (R)	0–1 (R)	0–1 (R)	0 (R)	0–1 (R)	0–1 (R)	1–2 (R)	0–1 (R)	1–2 (R)	3–4 (S)
Cultures 1–25	0–1 (R)	0–2 (R)	0–1 (R)	0 (R)	0–1 (R)	0–2 (R)	0–2 (R)	0–1 (R)	0–2 (R)	2–4 (S)
Culture 96/M–1	4 (S)	1–2 (R)	4 (S)	0 (R)	4 (S)	4 (S)	1–2 (R)	4 (S)	4 (S)	1–2 (R)

(R) = resistant reaction; (S) = susceptible reaction.

depend ultimately on mutation for the supply of new pathogenicity factors, and it is in this light that the significance of mutation in a rust such as *P. coronata avenae* must be assessed.

The experiments reported here demonstrate that mutation of genes controlling the pathogenic reaction of this rust can be induced by mutagenic treatment. Although a more extensive investigation involving a whole range of physiologic races would be required to determine the relative mutability of these genes, the fact that this mutant was obtained at the first attempt suggests a fairly high mutability. The similarity of the pathogenicity spectrum of the mutant to that of an existing race (277) demonstrates that the net results of induced and natural mutation are comparable. Since race 277 has been reported only in Argentina and Israel, the possibility that these results represent not a genuine mutation but a chance contaminant is extremely remote.

Two aspects of the data suggest that Flor's complementary gene hypothesis, as evolved for flax rust, is unlikely to be applicable to the host/parasite interaction of *P. coronata avenae*. First, the six cultivars to which the mutant was virulent, and to which the original race 96 was avirulent, possess different factors for resistance. Secondly, the pathogenic reaction was reversed on the diploid cultivar Saia, race 96 being virulent and the mutant avirulent. Thus Flor's hypothesis, which specifies a complementary gene for pathogenicity for every race-specific resistance gene in the host, is untenable here unless it is assumed that mutation at more than one locus is involved. Elucidation of this point would require the study of segregation of factors controlling virulence, following crossing and selfing of the mutant and race 96 on the alternate host.

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BRITISH RECORDS

58. *Pollaccia radiosa* (Lib.) Bald. & Cif. (*Atti Ist. bot. Univ. Pavia*, **4**, 10, p. 61, 1939) was collected on living leaves of *Populus tremula*, 27 May 1961 at Studland Dune Woods, Studland Bay, Dorset (Herb. I.M.I. 86897). Lesions 1–2 cm. diam., amphigenous, circular or more frequently irregular, occasionally confluent, cinnamon buff above, abruptly merging into a dull purplish-black margin 0·1–0·6 cm. wide, cream buff below with a dull purplish-black margin. Sporodochia amphigenous, following the veins when young, later deep olive and abundant above, sparse below. Mycelium immersed in the substratum, composed of branched, septate, hyaline or subhyaline, smooth-walled hyphae 1–3 μ wide. Stromata epidermal or subepidermal, poorly developed. Conidiophores singly erumpent through the epidermis or in groups of 2–8 arising from the upper cells of small stromata, cylindrical or obpyriform, straight, subhyaline to hyaline, 8–17 μ long × 3–5 μ thick, with few inconspicuous terminal annellations. Conidia formed singly at the apex of each conidiophore, which, after the first conidium has fallen, proliferates through the scar and forms a conidium at a higher level, ellipsoid or clavate, symmetrical, truncate at the base, subhyaline to ochraceous buff, smooth-walled with one distal

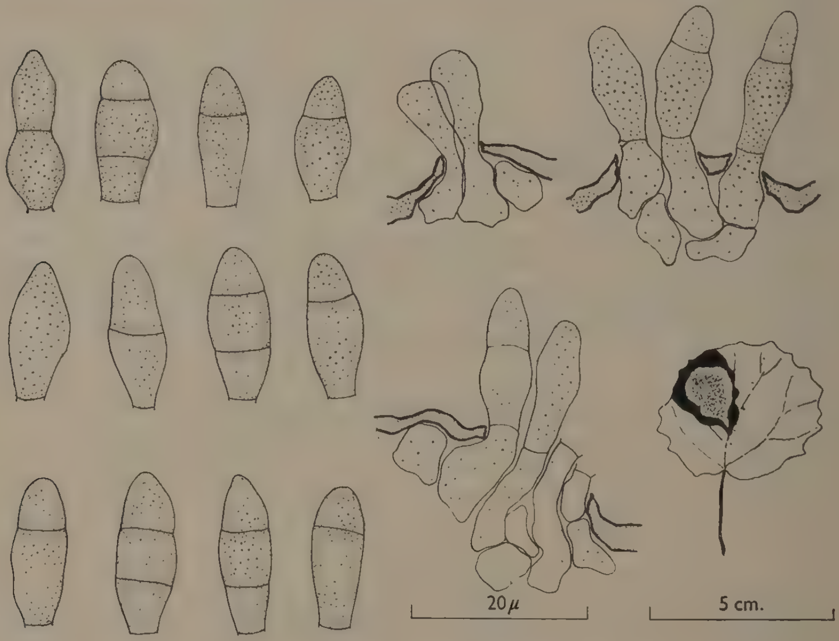

Fig. 1. *Pollaccia radiosa*, conidia, sporodochia and lesion.

transverse septum or two equidistant septa, slightly constricted, base furnished with a marginal frill, 14·5–19 × 6–7·5 μ. The collection was made from a small, isolated aspen wood at Studland Bay, it being the only wood of this type in that particular area. The small conidia and few annellations indicate that the material is rather young but the measurements, morphology and symptoms compare well with published exsiccatae from Hungary, Denmark, Canada and U.S.A., and agree closely with material collected in the same month from Germany (Herb. I.M.I. 7599). Peace (*Bull. For. Comm., Lond.*, **19**, p. 41, 1952) states that this fungus is doubtfully recorded from G.B. associated with die-back of aspen but D. M. Henderson (Edinburgh) has collected perfect and imperfect states of the fungus in Scotland. The species appears to be unrecorded from England. B. C. SUTTON, *Commonwealth Mycological Institute, Kew*

59. *Sporormia corynespora* Niessl, *Öst. bot. Z.* **28**, 45, 166, 1878, appeared on rabbit pellets from Wooltack Point, Pembroke, Wales (Fig. 2). It agrees with the original description and also with that of Griffiths (*Mem. Torrey bot. Cl.* **11**, 1901) except that the enlarged cell of the ascospore (the third from the upper end) is not usually clearly marked. The dark brown, almost black, spores are 8-celled, 54·5 × 12·8 μ (mean of 100 discharged spores selected at random). These values agree with those of Niessl (45–60 × 10μ), Griffiths (50–56 × 9–10 μ), Griffiths's Iowa specimen (45 × 8 μ), and Cane (*Studies of coprophilous Sphaeriales*, University of Toronto, 1934) (48–58 × 11–12 μ).

The old discharged spores break up into eight separate cells on drying. The spores are difficult to germinate but pure cultures have been obtained and deposited at the Commonwealth Mycological Institute (Herb. I.M.I. 86682). BRENDA BOOTH, WINIFRED PAGE, *Birkbeck College*

60. **Melastiza scotica** sp.nov.

Apothecia primum profunde concava dein patellaria, 10–25 mm. lata, sessilia, gregaria, margine breviter (*ca.* 500 μ) brunneo-pilosa, extra pallide aurantiaca et sat laxe pilis pallidioribus vestita, hymenio aurantiaco. Sporae 23–28 × 12·5–14 μ, anguste ellipsoideae, guttula unica vel guttulis binis majoribus instructae, grosse obtuse verrucosae, verrucae ad 3 μ altae, illae polos utrosque obtegentes ad 6 μ. Asci 380–400 × *ca.* 18 μ, cylindrici, octospori, sporis monostichis. Paraphyses filiformes apice ad 6 μ globoso-incrassata ac ibi intus granulis aurantiacis praedita. Pili ad 600 μ, e basi 15–20 μ crassa sensim attenuati ad apice obtuse rotundati, 4–6 cellulares, tenuiter tunicati, pallide brunneoli. Inter sphagna in pinetis, Cannich, Inverness, Sept. 1957, leg. R. Watling (typus in Herb. W.D.G. no. 1227).

The following collections have also been studied: (*a*) in conifer plantation, Porlock, Somerset, leg. D. A. & P. M. Reid, Aug. 1954; (*b*) on peaty soil, Killakee, Co. Dublin, leg. F. C. Hassall, Nov. 1955; (*c*) amongst pine debris, Rothiemurchus, Inverness, leg. D. A. Reid, Sept. 1957 (all in Herb. Kew); (*d*) in pine litter, Rothiemurchus, leg. P. D. Orton, Aug. 1960 (Herb. W.D.G. no. 1451); and (*e*) amongst moss in pine wood, Rannoch, Perthshire, leg. P. D. Orton, Oct. 1960 (Herb. W.D.G. no. 1467).

The apothecia tend to grow in tightly packed groups often somewhat immersed in the substratum, which included peat and pine needles in all collections. The marginal hairs are densely serried and vary from almost hyaline to clear brown; they originate from the excipular cells and have bluntly rounded tips about 7 μ thick; shorter similar hairs clothe the exterior. The remarkable spores are ornamented with enormous rounded warts up to 3 μ high and carry even larger irregularly shaped polar masses

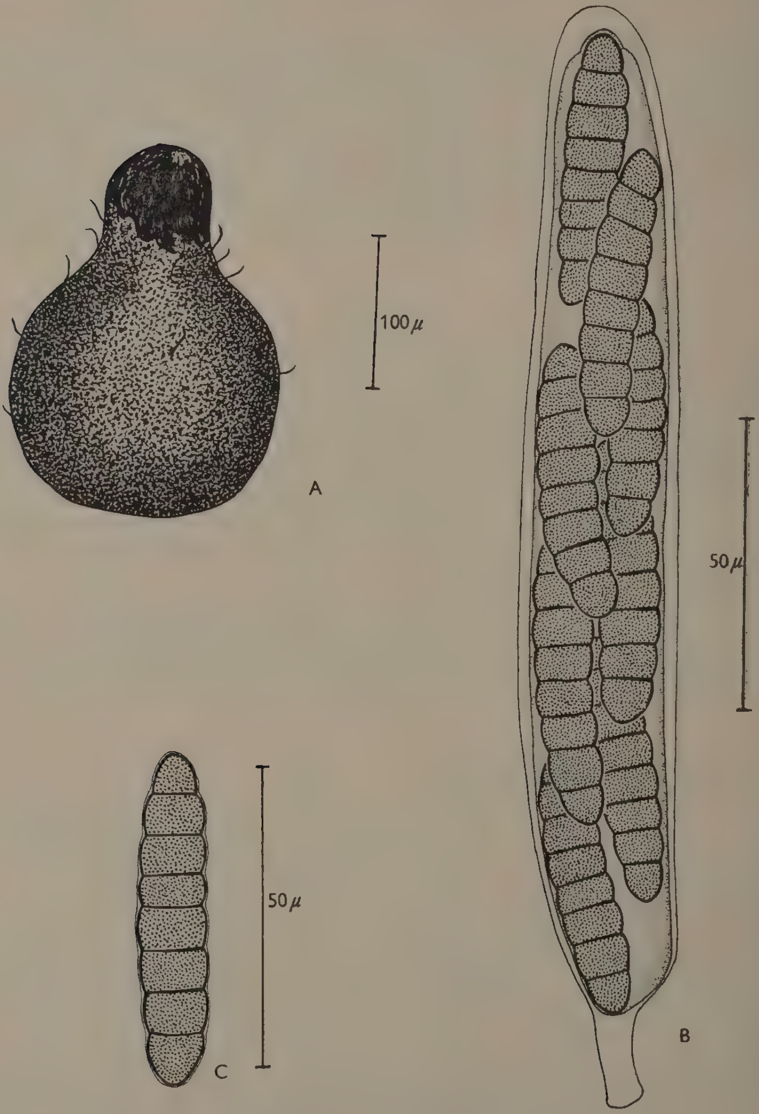

Fig. 2. *Sporormia corynespora.* A, perithecium; B, ascus; C, spore.

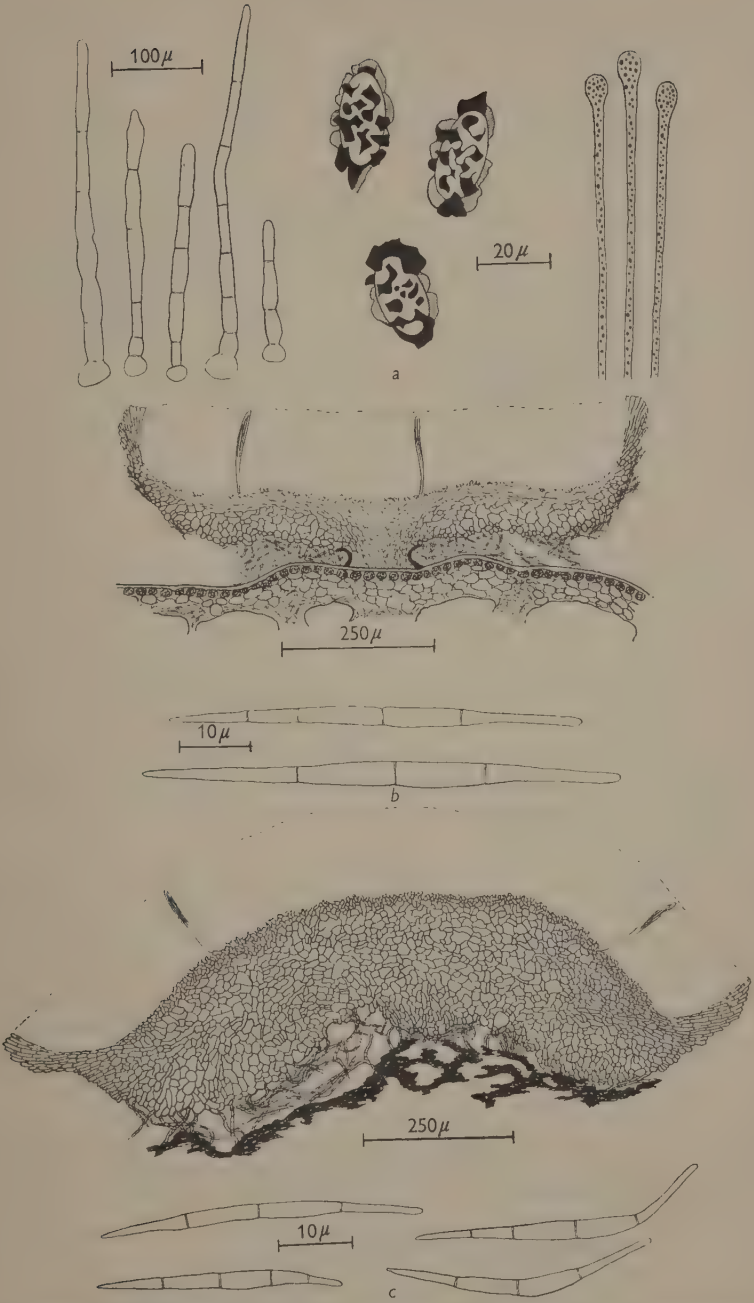

Fig. 3. *a*, *Melastiza scotica*. Hairs, spores and paraphyses. *b*, *Belonopsis iridis*. Spores and median section of apothecium. *c*, *B. lacustris* var. *caricinum*. Spores and median section of apothecium.

which may project as much as 6 μ; traces of a coarse reticulation can be seen on the spore surface between the warts. The paraphyses are distinctive in their more or less globose heads perched on slender rigid filaments (Fig. 3*a*).

I have some hesitancy in describing this fungus as new for it would seem that a relatively large species with such outstanding features and of which so many collections have recently become available must have already been described—but any such description has eluded me.

Clearly the fungus is near to *Melastiza boudieri* (Höhn.) Le Gal (*Bull. Soc. mycol. Fr.* **74**, 149–154, 1958) from which it differs in having larger and more strongly ornamented spores, much longer hairs and an orange hymenium instead of 'scarlet blood-red'. Moreover, our fungus is one of peaty soil whereas *M. boudieri* was collected on clay.

61. **Belonopsis iridis** (Crouan) comb.nov. (*Helotium iridis* Crouan, *Flore du Finistere*, p. 48). Scattered along stems of *Juncus* and leaves of *Typha* in Newtondale, Pickering, Yorks., leg. W. G. Bramley, June 1960 (Herb. W.D.G. no. 1435 and Herb. Kew). Discoid 1–5 mm. across, typically mollisioid in appearance. Spores 44–65 × 2·5–3 μ, one end acutely pointed the other narrowly cylindrical, with 3 or 5 septa the former greatly in excess.

This fungus has recently been described and illustrated by Mme Le Gal (*Rev. Mycol, Paris*, **18**, pp. 118–122, 1953) from material on *Juncus* in the Crouan herbarium and I now add to her figures one showing a median section of an apothecium (Fig. 3*b*); this shows a small footstalk and a strongly developed excipulum of thin-walled hyaline cells, from which the cortical layer of brown cells so commonly present in Mollisioideae is almost completely absent (Fig. 3*b*). For the present I think it expedient to retain species with long, multi-septate spores in *Belonopsis* (Sacc.) Rehm as interpreted by Nannfeldt (1932) rather than to disperse them within the vast assemblage of *Mollisia*.

62. *Belonopsis lacustris* (Fr.) Höhn. var. *caricinum* Vel. (Monograph of the Discomycetes of Bohemia, p. 128). Plentiful on leaf sheaths of *Carex flacca* on waste ground by the A 483 road about 1 m. N. of the Montgomery–Radnor boundary, May 1960 (Herb. W.D.G., no. 1434). Apothecia to 3 mm. across, growing singly or in groups of two to five; only at the very base of the host plant and consequently tedious to find. Spores 32–50 × 2–3 μ, one end narrowly pointed, the other pointed or narrowly cylindrical; 3-septate or occasionally asymmetrically 4-septate by division of one of the apical cells. Asci 125–150 × *ca.* 7 μ, pore blue in Melzer. Paraphyses very slender, only 3 μ at the rounded apex, many branched in lower half. Flesh thick, of hyaline parenchyma throughout except for a thin layer of minute-celled subhymenium. The similarity of hosts and the good agreement of hymenial details indicate the likely identity of this collection with Velenovsky's meagrely described fungus (Fig. 3*c*).

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63. *Monilinia johnsonii* (Ell. & Ev.) Honey (Dennis, British Helotiaceae, *Mycol. Pap.* **62**, p. 142, 1956). Syn. *Sclerotinia crataegi* Magnus (*Ber. dtsch. bot. Ges.* **23**, pp. 197–202, 1905).

During March and April 1961, apothecia developed in abundance on the mummified fallen fruits of a single scarlet hawthorn, *Crataegus oxyacanthoides*, in my garden in Abbots Leigh, Somerset. A disfiguring out-

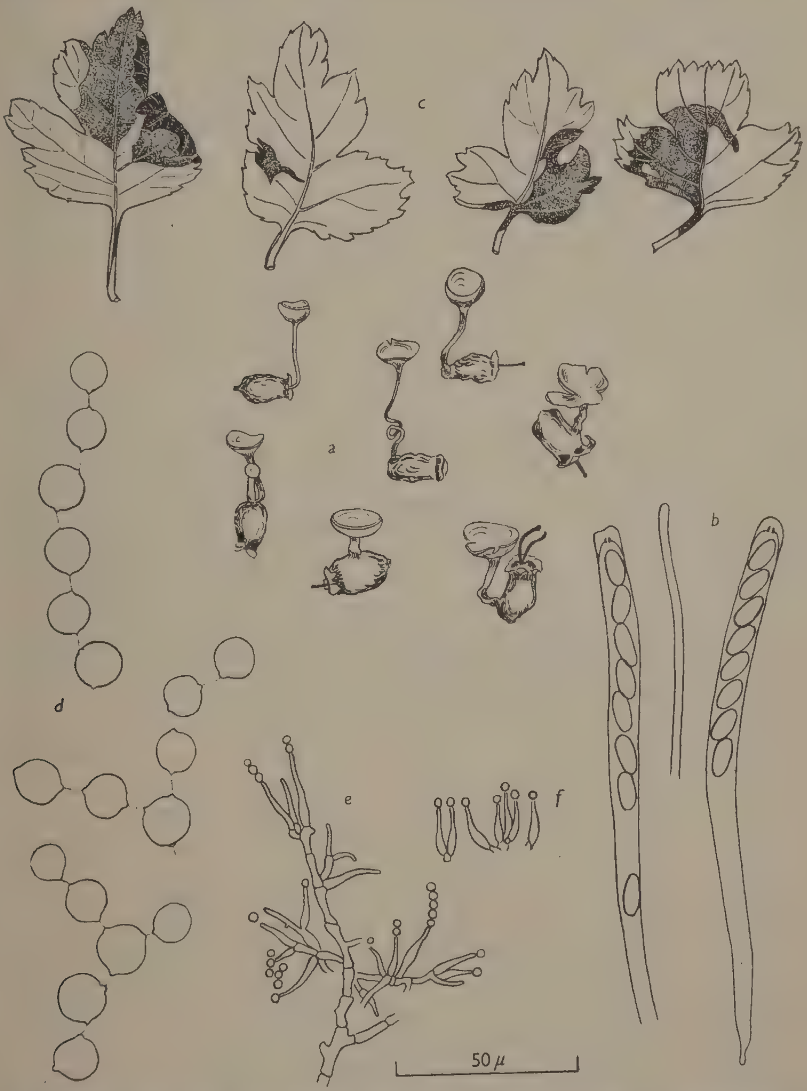

Fig. 4. *Monilinia johnsonii. a*, Apothecia arising from mummified fruits of *C. oxyacanthoides* (nat. size); *b*, asci and paraphysis, mounted in Melzer's reagent (× 500); *c*, early stages in development of macroconidial (leaf blotch) infection of foliage (nat. size); *d*, chains of macroconidia, showing disjunctors (× 500); *e*, *f*, production of phialides bearing microconidia. *e*, from 10-day culture on malt agar; *f*, from the surface of infected fruit (× 500).

break of the macroconidial leaf blotch disease had occurred on this tree in the previous spring and the foliage was again affected in the 1961 season. In both years leaf blotch was common on hedgerow trees of *C. monogyna* in the vicinity.

The apothecia (Fig. 4*a*), which have not previously been recorded in this country, were in close agreement with the original description and figure of Magnus. They arose singly or in pairs from all regions of the fruit but chiefly from the base—a count of 77 fruits gave 91 apothecia of which 71 were basal in origin. The light-brown disk measured 3·5–10 mm. diam., the stalk 2·0–19·5 mm. in length. The receptacle externally was dull reddish brown, smooth or appearing very slightly scurfy, sometimes with a scanty mycelial weft round the base of the stalk. The asci (Fig. 4*b*), strongly thickened at the apex and with the pore staining blue in Melzer's reagent, measured *ca.* 150–170 × 8 μ; the obliquely uniseriate, ellipsoid or slightly bean-shaped ascospores 10·5–14 × 5–6 μ.

Infection of the foliage, initially, is by air-borne ascospores, though the irregular outline of the affected areas in the early stages suggests a further limited spread of inoculum in the form of a spore suspension in the surface moisture on the leaf (Fig. 4 *c*). The leaf blotch, spreading to affect the greater part of the leaf lamina, usually stops short of the base of the petiole but occasionally it extends down the full length of the petiole and encircles the stem so that a 'twig blight' ensues. Typical diseased branches, with the blackened leaves hanging from the shoots, were figured by W. J. Dowson & W. A. R. Dillon Weston (*Gdnrs' Chron.* **101**, 426, 1937). Under favourable conditions the leaf blotches become thickly coated with a greyish to buff-coloured mould consisting of branched chains of subspherical, apiculate, hyaline conidia separated by exceedingly tenuous disjunctors (Fig. 4*d*). In the material examined, the conidia measured 10–13 × 8·5–13 μ. The diseased leaves emit a strong sweet smell, and the conidia are said to be transferred to the stigmas through the agency of insects.

On malt agar the conidia germinated to form masses of microconidia produced by typical phialides on a rather restricted aerial mycelium (Fig. 4*e*).

The infected fruits begin to turn brown while still on the tree, though most have fallen by the end of June. At this stage they are only a little smaller than the healthy fruits. After falling they shrivel slightly and become whitish or silvery in patches. When collected from the ground in July and kept moist for a day or two they became covered with a film of microconidia (spermatia) 2–3 μ in diameter, produced from densely tufted phialides (Fig. 4*f*). Fruit infection was virtually 100 % on the tree in my garden in 1960; in 1961, when the leaf blotch outbreak was judged to be considerably less severe, having been arrested by the onset of dry weather, it was estimated that 92 % of the fruits were infected. Of twenty current season's infected fruits collected from the ground in November 1960 and buried to their own depth in John Innes compost for overwintering in a plunge bed outside, nine had produced apothecia by the end of March 1961.

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REVIEWS

Handbuch für Pilzfreunde. Zweiter Band. *Nichtblätterpilze.* By EDMUND MICHAEL, revised by BRUNO HENNIG. (Jena: Gustav Fischer Verlag, 1960.) Pp. vi. + 328, 300 col. figures, 26 pl. and fig. Price: DM. 38.70.

This second volume of the promised four-volume work, started in 1958 (these *Transactions*, **41**, p. 523), is a mixed bag, containing all the non-agaricaceous, common, 'larger' basidiomycetes, the 'larger' ascomycetes, and eight myxomycetes, the last unfortunately sandwiched among the ascomycetes with no distinguishing heading. The illustrated part follows the same plan as before, but there is a long introductory section (27 chapters, 188 pp.) giving accounts of the mode of life of each group, structure and classification, with descriptions of and, in some instances, distinctions between the genera, though there are no keys as such, except one to the earth stars.

More species are depicted in colour than in Wakefield & Dennis, but that seems to be the only advantage to the British mycologist. The same band of artists has provided the paintings for the plates, the majority of which are good.

G. M. WATERHOUSE

The Biology of Fungi. By C. T. INGOLD. (London: Hutchinson Educational, 1961.) Pp. 124, 61 text-figures. Price: 12*s.* 6*d.*

This little book, the first Hutchinson Biological Monograph, written on the Atlantic, should bring a fresh mycological breeze into the classroom and blow in a number of facts which would not normally reach textbooks for another decade. The book is, to one reader at least, slightly disappointing, for after a promising title, a preface which sets mycological taxonomists and biochemists in perspective, the author finds himself beginning the seventh and final chapter (on the ecology of fungi) with the statement 'In the preceding chapters the fungi have been introduced mainly in a taxonomic manner, with digressions to consider questions of special biological interest'. And to push home the familiar taxonomic pattern there is a brief appendix outlining the scheme of classification of fungi. It is all rather too conventional, from Professor Ingold. Comprehension tests have, however, shown that the book will be warmly received as an introduction to fungi by both elementary students and others.

G. C. AINSWORTH

INDEX

Adams, K. F., *see* Hyde, H. A.
Agaricus bisporus, 285
Agnihothrudu, V. 'Notes on fungi from north-east India. V. A new genus of the helicosporous hyphomycetes', 51–54
Ainsworth, G. C., *see* Reviews
Air spora, 134–139, 298–300
Algae, marine, Didymosphaeria spp. on, 55
Anguillospora crassa, 559
Antibiotics, 145, 354, 365, 377
Aposphaeria violacea, 573
Apricot, freckle of, 337
Aquatic fungi, 27, 87, 91, 163, 177, 305, 559, 586
Armillaria mellea, 123
Aspergillus brunneo-uniseriatus, 160
  parvulus, 45
  restrictus, 45

Bakshi, B. K., *see* Singh, Sujan
Bamford, P. C., Norris, G. L. F. and Ward, G. 'Flavipin production by *Epicoccum* spp.', 354–356
Banana, fruit-spot ('speckle') of, 95, 265, 391
  leaf spot of, 299
  tip-rot of fruit of, 487
Barnett, H. L., *see* Reviews
Barton, R. 'Saprophytic activity of *Pythium mamillatum* in soils. II. Factors restricting *P. mamillatum* to pioneer colonization of substrates', 105–118
Batista, A. Chaves, *see* Reviews
Belonopsis iridis, 612
  lacustris, 612
Bevan, E. A., *see* Cox, B. S.
Beverwijk, A. L. van, *see* Reviews
Blastocladiella britannica, 185
Bond, T. E. T., *see* British Records
Booth, Brenda, *see* British Records
Booth, C. and Ebben, Marion H. '*Thielavia reticulata* sp.nov.', 214–216
  and Robertson, J. S. '*Leptosphaeria elaeidis* sp.nov. isolated from anthracnosed tissue of oil palm seedlings', 24–30
  see Reviews
Botryotrichum piluliferum, 79
Brassicae, club root of, 593
British Mycological Society:
  Annual General Meeting, 298
  Proceedings, 134–139, 298–300, 458–459
British Records, 608–614
Brown, W., *see* Reviews
Bunce, Maureen E., '*Humicola stellatus* sp.nov., a thermophilic mould from hay', 372–376
Bunting, A. H. 'Introduction. The leaf and its microflora', 298
Burnett, J. H. 'Mating types, individuals and populations in higher fungi', 459
Butler, G. M. 'Growth of vegetative hyphae in *Coprinus disseminatus* and other fungi', 458

Cacao, black pod disease of,
Canter, Hilda M. 'Studies on British chytrids. XVII. Species occurring on planktonic desmids', 163–176
  'XIX. On *Phlyctidium apophysatum* Canter emend.', 522–528
Cantino, E.C., see Horenstein, Evelyn A.
Carex spp., Stagonospora macropycnidia on, 87
Carlile, M.J., Lewis, B.G., Mordue, Elizabeth M. and Northover, J. 'The development of coremia. I. *Penicillium claviforme*', 129–133
Carr, A. J. H., *see* Griffiths, D. J.
Carré, C. G. and Harrison, R. W. 'Studies on vesicular-arbuscular endophytes. III. An endophyte of *Conocephalum conicum* (L). Dum identified with a strain of *Pythium*', 565–572
Cartter, Margaret A., *see* Macdonald, J. A.
Centenary, of Miss Gulielma Lister, 140
Chadefaud, M., *see* Reviews
Chaetomium fusisporum, 46
  piluliferum, 79
Chytriomyces poculatus, 177
Coffee, berry disease of, 511
Colhoun, John. 'Spore load, light intensity and plant nutrition as factors influencing the incidence of club root of Brassicae', 593–600
Colletotrichum coffeanum, 511
Conocephalum conicum, endophyte of, 565
Coprinus disseminatus, 458
  lagopus, 459
Coremia, 129
Corner, E. J. H. 'A note on *Wiesnerina* (Cyphellaceae)', 230–232
  and K. S. Thind 'Dimitic species of Ramaria (Clavariaceae)', 233–238
  see Reviews
Cox, B. S. and Bevan, E. A. 'A new technique for isolating spores of yeast (*Saccharomyces cerevisiae*), 239–242
Ctenomyces serratus, 449
Cunnell, G. J. '*Stagonospora macropycnidia* sp.nov.', 87–90
Curvularia ramosa, 145

Dade, H. A., *see* Reviews
Daniels, Joan. '*Chaetomium piluliferum* sp.nov., the perfect state of *Botryotrichum piluliferum*', 79–86
Day, P. R. 'Mating type genes in *Coprinus*', 459
Deightoniella torulosa, 95, 265, 391, 487
Dimond, A. E., *see* Reviews
Dog, otitis externa of, 441
Doling, D. A. 'West European yellow rust investigations', 134–135
Dring, D. M. 'Studies on *Mycosphaerella brassicicola* (Duby) Oudem.', 253–264
  '*Ramularia armoraciae* Fuckel', 333–336
Duddington, C. L., *see* Reviews

Ebben, Marion H., *see* Booth, C.
Eladia, 47
Ellis, M. B., *see* Reviews
Entophlyctis lobata, 177
Epichloe typhina, 493
Epicoccum, 354
Erwin, D. C., *see* Kennedy, B. W.
Evans, E. 'Relationship between the physical characteristics of a banana leaf and the distribution of leaf spot lesions (*Mycosphaerella musicola* Leach)', 299
Eveleigh, D. E. '*Phoma* spp. associated with painted surfaces', 573–585

Fisher, Eileen E. '*Venturia carpophila* sp.nov., the ascigerous state of the apricot freckle fungus', 337–342
Flavipin, 354
Flood, A. E. 'The biochemistry of the leaf in relation to disease', 300
Fomes annosus, 145
Fraser, Gordon. '*Pityrosporum pachydermatis* Weidman of canine origin', 441–448
Frey, Dorothea and Griffin, D. M. '*Ctenomyces serratus* Eidam', 449–452
Fusarium avenaceum, 145
  caeruleum, 145
  culmorum, 145, 343
  oxysporum, 119, 377
  oxysporum f. cubense, 145
  oxysporum f. vasinfectum, 145

Genetics of higher basidiomycetes, symposium on, 458
Gentles, J. C., *see* Reviews
Gibson, I. A. S. 'A note on variation between isolates of *Armillaria mellea* (Vahl ex Fr.) Kummer', 123–128
Gongronella butleri, 49
Good, H. M., *see* Grindle, Morris
Graddon, W. D., *see* British Records
Grasses, choke of, 493
Gray, James, *see* Reviews
Gregory, P. H. 'The leaf as a spore trap', 298–299
Griffin, D. M., *see* Frey, Dorothea
Griffiths, D. J. 'Breeding for resistance to crown rust in the oat crop', 134
  and Carr, A. J. H. 'Induced mutation for pathogenicity in *Puccinia coronata avenae*,' 601–607
Griffiths, Ellis and Siddiqi, M. A. 'Some factors affecting occurrence of *Fusarium culmorum* in the soil', 343–353
Grindle, Morris and Good, H. M. 'Effects of drying on the viability of germinated and germinating conidia of *Monilinia fructicola* (Wint.) Honey', 549–558

Harrison, R. W., *see* Carré, C. G.
Hawthorn, leaf blotch of, 613
Helicobasidium purpureum, 145
Heliscus submersus, 91
Helminthosporium phlei, 217
  sativum, 145
Hennig, Bruno, *see* Reviews
Hirst, J. M. 'The aerobiology of *Puccinia graminis* uredospores', 138–139
Hogg, W. H. 'Meteorology in relation to recent black rust epidemics', 137
Hora, F. B., *see* Reviews
Horenstein, Evelyn A. and Cantino, E. C. 'Morphogenesis in and the effect of light on *Blastocladiella britannica* sp.nov.', 185–198
Horsfall, J. G., *see* Reviews
Hudson, H. J. '*Heliscus submersus* sp.nov., an aquatic hyphomycete from Jamaica', 91–94
  '*Oncopodium panici* sp.nov., a hyphomycete from Jamaica', 406–408
Humicola stellatus, 372
Hyde, H. A. and Adams, K. F. 'Spore trapping in relation to recent black rust epidemics', 138

Ingold, C. T. 'Another aquatic spore-type with clamp connexions', 27–30
  see Reviews

Jarvis, W. R. 'Growth of isolates of *Phytophthora fragariae* Hickman in the presence of various polyphenols', 357–364

Karling, John S., '*Nowakowskiella sculptura* sp.nov.', 453–457
Kennedy, B. W. and Erwin, D. C. 'Some factors influencing sporangium formation of a *Phytophthora* species isolated from lucerne in certain salt solutions', 291–297
Kerr, A. 'A study of tomato root surface organisms antagonistic to *Verticillium albo-atrum*', 365–371
Kerr, Janet E. 'The life history and taxonomic position of *Venturia rumicis* (Desm.) Wint.', 465–486
Kirby, E. J. M. 'Host-parasite relations in the choke disease of grasses', 493–503
Knoyle, J. Mary, *see* Wilson, Irene M.

La Touche, C. J., *see* Reviews
Lapwood, D. H. 'Varietal differences in the susceptibility of potato leaves to blight', 300
Large, E. C. 'Presidential address. Pursuits of mycology', 1–23
Last, F. T. 'The saprophytic flora of the phyllosphere', 300
Leaf microfloras, 298
Leptosphaeria elaeidis, 24
Lewis, B. G. *see* Carlile, M. J.
Lewis, D. 'The mycelium as a biochemical and genetical unit', 458–459
Light, effect on fungal morphogenesis, 129
Lister, Miss Gulielma, centenary of, 140
Lucas, Maria T., *see* Webster, John
Lythgoe, J. N. 'Effect of light and temperature on growth and development in *Thamnidium elegans* Link', 199–213

Macdonald, J. A. and Cartter, Margaret A. 'The rhizomorphs of *Marasmius androsaceus* Fries', 72–78

Macer, R. C. F. 'Breeding for resistance to yellow rust in wheat', 135
Macrophomina phaseoli, 145
Mantle, P. G. 'Further observations on an abnormal reaction of wheat to loose smut', 529–545
  'Persistence of loose smut infection within the growing point region of susceptible wheat varieties', 546–548
Marasmius androsaceus, 72
Martin, Jeanne, *see* Reviews
Martin, J. T. 'The leaf cuticle in relation to fungal invasion', 299
Mathew, K. T. 'Morphogenesis of mycelial strands in the cultivated mushroom, *Agaricus bisporus*', 285–290
Maublanc, A., *see* Reviews
Melastiza scotica, 609
Meredith, D. S. 'Fruit-spot ('speckle') of Jamaican bananas caused by *Deightoniella torulosa* (Syd.) Ellis. I. Symptoms of disease and studies on pathogenicity', 95–104
  'II. Factors affecting spore germination and infection', 265–284
  'III. Spore formation, liberation and dispersal', 391–405
  '*Deightoniella torulosa* (Syd.) Ellis and *Verticillium theobromae* (Turc.) Mason & Hughes associated with a tip-rot of banana fruits', 487–492
Michael, Edmund, *see* Reviews
Mollisia, 559
Monilinia fructicola, 549
  johnsonii, 613
Mordue, Elizabeth M., *see* Carlile, M. J.
Morphogenesis, 129, 185, 199, 285, 377, 458
Mortierella, 243
Mucor vesiculosus, 49
Mutation for pathogenicity, induced, 601
Mycorrhiza, vesicular-arbuscular, 565
Mycosphaerella brassicicola, 253
  maculiformis, 465
  musicola, 299
Myrothecium, 31

New fungal genera, 47, 53, 437
  species, 24, 42, 53, 79, 87, 91, 160, 163, 177, 185, 214, 337, 372, 406, 431, 433, 437, 453, 573, 586, 609
  varieties, 427
New taxonomic combinations, 57, 217, 375, 439, 577, 612
Norris, G. L. F., *see* Bamford, P. C.
Northover, J., *see* Carlile, M. J.
Nowakowskiella sculptura, 453
Nutman, F. J. and Roberts, F. M. 'Investigations on a disease of *Coffea arabica* caused by a form of *Colletotrichum coffeanum* Noack. III. The relation between infection of bearing wood and disease incidence', 511–521

Oats, crown rust of, 134, 601
  take-all of, 504
Obelidium megarhizum, 586
Ogilvie, L. 'The West European and British black rust investigations', 136
Oil palm, anthracnose of, 24
Oncopodium panici, 406
Ophiobolus graminis, 145, 504
  var. avenae, 504

Page, Winifred, *see* British Records
Paint films, spoilage of by Phoma spp., 573
Park, David. 'Isolation of *Fusarium oxysporum* from soils', 119–122
  'Morphogenesis, fungistasis and cultural staling in *Fusarium oxysporum* Snyder & Hansen', 377–390
  see Reviews
Parkinson, D., *see* Reviews
Parrot, A. G., *see* Reviews
Penicillium claviforme, 129
  kewense, 42
  kojigenum, 43
  madriti, 44
Phlyctidium apophysatum, 522
Phlyctochytrium aureliae, 586
  planicorne, 163
Phoma saprophytica, 573
  violacea, 577
Phytophthora fragariae, 357
  infestans, 300
  palmivora, 409
  species isolated from lucerne, 291
Picken, L. E. R., *see* Reviews
Pityrosporum pachydermatis, 441
Planktonic desmids, chytrids parasitic on, 163
Plasmodiophora brassicae, 593
Pleospora, 417, 465
Plunkett, B. E. 'Tropisms and formageniture in sporophores of *Polyporus brumalis*', 458
Podochytrium chitinophilum, 586
Pollaccia radiosa, 608
Polypaecilum, 437
Polyporus brumalis, 458
Potato, blight of, 300
Preston, N. C. 'Observations on the genus *Myrothecium*. III. The cylindrical-spored species of *Myrothecium* known in Britain', 31–41
Puccinia coronata avenae, 134, 601
  glumarum, 134, 135
  graminis tritici, 136–139
  striiformis, *see* Puccinia glumarum
Pugh, G. J. F., *see* Turner, Myfanwy
Pythium mamillatum, 105, 145
  sp., as endophyte of Conocephalum conicum, 565

Ramaria, 233
Ramularia armoraciae, 333
Raper, J. R. 'An inquiring look at tetrapolar incompatibility', 459
Reviews:
  Anon., Herb. I.M.I. Handbook, 1960 (by A. L. van Beverwijk), 141
  Barnett, H. L., Illustrated genera of imperfect fungi, 1960 (by M. B. Ellis), 143
  Batista, A. Chaves, Monografia dos fungos Micropeltaceae, 1959 (by C. Booth), 304

Reviews (*continued*)
  Chadefaud, M., Traité de botanique systématique. Tome I. Les végétaux non vasculaires (Cryptogamie), 1960 (by E. J. H. Corner), 461
  Duddington, C. L., Practical microscopy, 1960 (by H. A. Dade), 143; Micro-organisms as allies, 1961 (by Jeanne Martin), 462
  Gentles, J. C. (ed.), Sabouraudia, vol. 1, 1961 (by C. J. La Touche), 460
  Horsfall, J. G. and Dimond, A. E. (ed.), Plant pathology. An advanced treatise. Vol. II. The pathogen, 1960 (by W. Brown), 301
  Ingold, C. T., The biology of fungi, 1961 (by G. C. Ainsworth), 615
  Maublanc, A., Champignons de France, 1960 (by F. B. Hora), 144
  Michael, Edmund, Handbuch für Pilzfreunde. Band II. Nichtblätterpilze (revised by Bruno Hennig), 1960 (by G. M. Waterhouse), 615
  Parkinson, D. and Waid, J. S. (ed.), The ecology of soil fungi. An international symposium, 1960 (by D. Park), 142
  Parrot, A. G., Amanites du sud-ouest de la France, 1960 (by G. M. Waterhouse), 144
  Picken, L. E. R., The organization of cells and other organisms, 1960 (by James Gray), 462
  Sparrow, F. K. Aquatic phycomycetes, 1960 (by G. M. Waterhouse), 304
Rhizoctonia solani, 145
Rhizomorphs, 72, 123
Rhizophydium chaetiferum, 163
  gibbosum, 163
  ubiquetum, 163
Roberts, F. M., *see* Nutman, F. J.
Robertson, J. S., *see* Booth, C.

Saccharomyces cerevisiae, 239
Salt marsh, Mortierella spp. from, 243
Saprophytic ability, competitive, 105, 145
Scharif, G. 'Studies on graminicolous species of *Helminthosporium*. I. *H. phlei* (Graham) comb.nov', 217–229
Siddiqi, M. A., *see* Griffiths, Ellis
Sing, Sujan and Bakshi, B. K. 'A new species of *Aspergillus*', 160–162
Smith, George. 'Some new and interesting species of micro-fungi. II', 42–50
  '*Polypaecilum* gen.nov.', 437–440
Smith, R. S. 'Uredospore dissemination and infection', 136
Soil fungi, 105, 119, 305, 343, 453
Sparrow, F. K., *see* Reviews
Sporormia corynespora, 609
Stagonospora macropycnidia, 87
Staling of fungal cultures, 377
Sutton, B. C., *see* British Records
Symposia, 134, 298, 458

Techniques, new. 119, 239, 549
Temperature, effect on fungal morphogenesis, 199
Thamnidium elegans, 199
Thermophilic fungi, 372
Thielavia reticulata, 214
Thind, K. S., *see* Corner, E. J. H.
Thorpe, I. G. 'Recent work at Bristol on black rust epidemics', 137
Tomato, root surface organisms of, 365
Townley, Patricia J., *see* Willoughby, L. G.
Turner, Myfanwy and Pugh, G. J. F. 'Species of *Mortierella* from a salt marsh', 243–252
Turner, P. D. 'Strains of *Phytophthora palmivora* (Butl.) Butl. from *Theobroma cacao* L. II. Isolates from non-African countries', 409–416
Typha latifolia, Stagonospora macropycnidia on, 87

Ustilago nuda, 529, 546

Vanbeverwijkia, 53
Venturia carpophila, 337
  inaequalis, 465
  pirina, 465
  rumicis, 465
Verticillium albo-atrum, 365
  dahliae, 145
  theobromae, 487

Waid, J. S., *see* Reviews
Ward, G., *see* Bamford, P. C.
Wastie, R. L. 'Factors affecting competitive saprophytic colonization of the agar plate by various root-infecting fungi', 145–159
Waterhouse, G. M., *see* Reviews
Webster, John. 'The *Mollisia* perfect state of *Anguillospora crassa*', 559–564
  and Lucas, Maria T. 'Observations on British species of *Pleospora*. II', 417–436
Wheat, loose smut of, 529, 546
  stem rust of, 136–139
  take-all of, 504
  yellow rust of, 134, 135
Wiesnerina, 230
Willetts, H. J. 'A comparison between *Ophiobolus graminis* and *Ophiobolus graminis* var. *avenae*', 504–510
Willoughby, L. G. 'The ecology of some lower fungi at Esthwaite Water', 305–332
Willoughby, L. G. 'Chitinophilic chytrids from lake muds', 586–592
  and Townley, Patricia J. 'Two new saprophytic chytrids from the Lake District', 177–184
Wilson, Irene M. and Knoyle, J. Mary. 'Three species of *Didymosphaeria* on marine algae: *D. danica* (Berlese) comb. nov., *D. pelvetiana* Suth. and *D. fucicola* Suth.', 55–71
Wood, R. K. S., *see* Proceedings, 300

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CONTENTS

PAGE

The life history and taxonomic position of *Venturia rumicis* (Desm.) Wint. By Janet E. Kerr. (With 5 Text-figures) 465

Deightoniella torulosa (Syd.) Ellis and *Verticillium theobromae* (Turc.) Mason & Hughes associated with a tip-rot of banana fruits. By D. S. Meredith. (With Plate 31 and 5 Text-figures) 487

Host–parasite relations in the choke disease of grasses. By E. J. M. Kirby. (With Plates 32 and 33 and 3 Text-figures) 493

A comparison between *Ophiobolus graminis* and *Ophiobolus graminis* var. *avenae*. By H. J. Willetts 504

Investigations on a disease of *Coffea arabica* caused by a form of *Colletotrichum coffeanum* Noack. III. The relation between infection of bearing wood and disease incidence. By F. J. Nutman and F. M. Roberts. (With 9 Text-figures) 511

Studies on British chytrids. XIX. On *Phlyctidium apophysatum* Canter emend. By Hilda M. Canter. (With Plate 34 and 3 Text-figures) 522

Further observations on an abnormal reaction of wheat to loose smut. By P. G. Mantle. (With Plate 35 and 2 Text-figures) 529

Persistence of loose smut infection within the growing point region of susceptible wheat varieties. By P. G. Mantle. 546

Effects of drying on the viability of germinated and germinating conidia of *Monilinia fructicola* (Wint.) Honey. By Morris Grindle and H. M. Good. (With Plate 36 and 2 Text-figures) 549

The *Mollisia* perfect state of *Anguillospora crassa*. By John Webster. (With 2 Text-figures) 559

Studies on vesicular-arbuscular endophytes. III. An endophyte of *Conocephalum conicum* (L.) Dum identified with a strain of *Pythium*. By C. G. Carré and R. W. Harrison. (With 1 Text-figure) 565

Phoma spp. associated with painted surfaces. By D. E. Eveleigh 573

Chitinophilic chytrids from lake muds. By L. G. Willoughby. (With Plates 37 and 38 and 2 Text-figures) 586

Spore load, light intensity and plant nutrition as factors influencing the incidence of club root of Brassicae. By John Colhoun 593

Induced mutation for pathogenicity in *Puccinia coronata avenae*. By D. J. Griffiths and A. J. H. Carr. (With 1 Text-figure) 601

British Records 608

Reviews 615

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